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PREDICTING PROSTATE CANCER

On the use of biomarkers in prostate cancer diagnostics

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ABSTRACT

Aims

The aims of this thesis work were to answer the following questions. *Paper I*: How prevalent is testing and retesting with prostate-specific antigen (PSA)?; *Paper II*: Is a genetic score based on single-nucleotide polymorphisms (SNPs) informative regarding the risk of prostate cancer (PCa) in men with low PSA?; *Paper III*: Are the commercially available tests Prostate Health Index (PHI) and the four-kallikrein panel comparable in aiding biopsy decisions?; *Paper IV*: Do commonly used medications affect PSA and the risk of PCa?

Methods

In *Paper I* and *Paper IV*, the population-based PSA cohort STHLM0 was used together with registry-based data. *Paper I* described limited-duration point prevalence of testing and survival analysis describing retesting with PSA. *Paper IV* determined differences in PCa risk and PSA level among men using aspirin, statin, metformin or no medication. *Paper II* included 172 men with PSA at 1–3ng/ml. Participants were invited according to their genetic score and underwent prostate biopsy. Risk of prostate cancer was assessed using logistic regression. *Paper III* included 531 men who had undergone a first prostate biopsy. Predictive models were compared using receiver-operating characteristics (ROC/AUC) and calculation of biopsies that could be avoided.

Results

Paper I: During a 9-year study period, 46%, 68%, and 77% of men without previous PCa and aged 50–59 years, 60–69 years, and 70–79 years, respectively, had a PSA test. The probability of retesting with PSA was PSA- and age-dependent, with a 26-month cumulative incidence of 0.34 if the first PSA value was < 1 ng/ml. *Paper II*: PCa was diagnosed in 47 of 172 men with PSA levels of 1–3ng/ml (27%), with Gleason sum of ≥ 7 in 10 of them (5.8%). There was an increase in the odds ratio of 1.60 with increasing genetic risk score. The absolute difference in risk of positive biopsy was 19 percentage points, comparing the high and low genetic risk groups (37% vs. 18%). *Paper III*: The four-kallikrein panel showed AUCs of 69.0 when predicting PCa of any grade and 71.8 when predicting high-grade cancer (Gleason score ≥ 7). Similar values were found for PHI (70.4 and 71.1, respectively). Both models had higher AUCs than a base model with PSA value and age. Using a 10% predicted risk of high-grade PCa by the four-kallikrein panel or PHI = 39 as cutoff for biopsy saved 29% of the biopsies performed, at a cost of delayed diagnosis for 10% of the men with high-grade cancer. *Paper IV*: There were no significant associations between aspirin or any anti-diabetic medication and the risk of PCa. Men using any statin had an increased risk of both high-grade PCa and PCa overall (OR = 1.25; OR = 1.16). Compared to men without the medication, the level of the first PSA was lower in men using aspirin, statin, metformin, or insulin.

Conclusions

Although screening for PCa is not recommended in Sweden, PSA testing in Stockholm County was high in men aged over 50 years. A risk score based on SNPs predicts biopsy outcome in previously unbiopsied men with PSA levels of 1–3 ng/ml. Furthermore, we found that two blood tests, the PHI and the four-kallikrein panel, performed similarly in predicting prostate biopsy outcome. Introduction of such risk stratification tools could increase the proportion of men being classified in line with their true risk of PCa. We found no protective effect of aspirin, statins, or antidiabetics in terms of overall risk of prostate cancer or high-grade cancer.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Prostatacancer är den vanligaste cancer-relaterade dödsorsaken hos svenska män och cirka 1 av 20 män avlider av sjukdomen. En ansenlig andel av män med icke avancerad prostatacancer påverkas dock inte av sjukdomen under sin livstid. Efter att testning med blodprovet Prostata-Specifikt Antigen (PSA) inleddes har förekomsten av känd prostatacancer ökat kraftigt, och dödligheten har minskat på senare år. Nuvarande utbredning av testning med PSA är ofullständigt känd.

PSA-testning kan leda till minskad risk att dö av prostatacancer, då man kan diagnosticera och kan behandla sjukdomen i ett tidigt skede. Å andra sidan har PSA-provet begränsad precision att identifiera män som kommer utveckla allvarlig sjukdom och man riskerar att diagnosticera många män med ofarlig sjukdom om organiserade screening-program införs. Därför är det prioriterat att utveckla och validera bättre testverktyg för att detektera potentiellt allvarlig prostatacancer. Särskilt bland män med hög risk för sjukdom är det rimligt att också utvärdera värdet av förebyggande medicinering. Flera mediciner har föreslagits som associerade med risken att utveckla prostatacancer, men ingen har hittills visat tillräckligt värde för att användas.

I den här avhandlingen presenteras fyra delarbeten med målsättningen att (i) kartlägga hur PSA-testning används idag, (ii) validera hur väl nya test-verktyg presterar i att förutspå om en man har prostatacancer och (iii) att studera om medicinerna acetyl-salicylsyra, statiner eller metformin har en skyddande effekt på risken att utveckla prostatacancer.

I **delarbete I** utnyttjades den befolkningsbaserade databasen STHLM0 som täcker alla män som genomgått PSA-testning i Stockholms län sedan 2011. Vi använde register-samkörningar med befolkningsregister och flera kvalitetsregister för att uppskatta både förekomsten av PSA-testning och återkommande testning. Vi fann att det var vanligt att genomgå PSA-test och att testning var vanlig även bland grupper som sannolikt inte gagnas av det. Cirka 6 av 10 män mellan 50 och 80 år genomgick testning med PSA de senaste 9 åren. Återkommande testning var vanlig även hos män med låga PSA-värden som har låg risk att utveckla allvarlig sjukdom. Vidare fann vi att det var vanligt att genomgå PSA-test även bland äldre män som har mycket lite att vinna på sådan testning.

En mans uppsättning av genetiska nukleotidpolymorfismer (SNP) är associerad med hans risk att utveckla prostatacancer. Denna kännedom är begränsad till män som utreds för prostatacancer i dagens vård, trots att vi vet att det förekommer prostatacancer även hos symtomfria män med låga PSA-värden. Målet med **delarbete II** var att studera sambandet mellan uppsättningen SNP hos män med låga PSA-värden och deras risk att ha prostatacancer. Vi genomförde en klinisk studie där vi bjöd in män till vävnadsprov av prostata. Vi fann att en mans uppsättning av SNP är associerat med risken att ha prostatacancer bland män med låga PSA-värden. Analys av SNP-uppsättning utgör en möjlighet att identifiera män med hög risk för prostatacancer som inte utreds enligt klinisk praxis idag.

Flera verktyg har föreslagits för att underlätta bedömningen av en mans risk att ha prostatacancer. Två sådana är de kommersiellt tillgängliga blodproven Prostate Health Index (PHI, Beckman Coulter Inc.) och four-kallikrein panel (4KScore, OPKO Health Inc.). I **delarbete III** jämförde vi hur väl dessa test presterade i en svensk grupp av män som genomgått utredning med vävnadsprover av prostata (STHLM2). Vi fann att PHI och 4KScore presterade likvärdigt och kunde bespara cirka tre av tio män vävnadsprovtagning enligt klinisk praxis till kostnaden av att missa ett av tio hög-gradiga cancerfall. Både PHI och 4KScore identifierade prostatacancer bättre än en modell med PSA och ålder.

För att säkerställa nyttan med en medicinsk intervention såsom screening eller erbjudande av förebyggande medicinering krävs ofta stora och kostsamma randomiserade prövningar. Epidemiologiska studier erbjuder ofta lägre värderad, men lättare tillgänglig kunskap och kan vägleda om stora prövningar ska inledas. **Delarbete IV** är en epidemiologisk studie där vi använde samma stora grupp män som i delarbete I. Vi identifierade män som genomgått PSA-provtagning och sedan män som genomgått vävnadsprovtagning av prostata. Vi samkörde studiegruppen mot bland annat läkemedelsregistret (Socialstyrelsen) för att utvärdera om medicinering med acetylsalicylsyra, stationer eller anti-diabetika såsom metformin var associerad med risken att finna prostatacancer vid vävnadsprovtagning. Vi fann inga tecken till någon skyddande effekt av acetylsalicylsyra, stationer eller metformin för risken att utveckla prostatacancer och planerar inte att ta initiativ till större prövningar för att studera förebyggande behandling med dessa mediciner.

LIST OF PUBLICATIONS

I. Nordström T, Aly M, Clements MS, Weibull CE, Adolfsson J, & Grönberg H
Prostate-specific Antigen (PSA) Testing Is Prevalent and Increasing in Stockholm County, Sweden, Despite No Recommendations for PSA Screening: Results from a Population-based Study, 2003–2011.
European Urology, 2013;*63*(3), 419–425.

Awarded Best Scientific Paper in European Urology by a resident in the year 2013/2014

Editorial and reply from authors
European Urology, 2013;63(3), 426-427

II. Nordström T, Aly M, Eklund M, Egevad L, & Grönberg H
A Genetic Score Can Identify Men at High Risk for Prostate Cancer Among Men With Prostate-Specific Antigen of 1–3 ng/ml.
European Urology, 2014; 65(6), 1184-1190

III. Nordström T, Vickers A, Assel M, Lilja H, Grönberg H, & Eklund M
Comparison between the four-kallikrein panel and Prostate Health Index (PHI) for predicting prostate cancer.
European Urology, 2014; E-pub ahead of print

IV. Nordström T, Clements MS, Karlsson R, Adolfsson J, & Grönberg H
The risk of cancer findings on prostate biopsy among men on aspirin, statin or antidiabetic medication.
Submitted

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LIST OF ABBREVIATIONS

PCa	Prostate cancer
DRE	Digital rectal examination
PSA	Prostate-specific antigen; human kallikrein 3; hK3; tPSA
fPSA	Free PSA
f/tPSA	fPSA/tPSA
iPSA	Intact PSA
bPSA	Benign PSA
hK2	Human kallikrein 2
PHI	Prostate Health Index
4K	Four-kallikrein panel; 4Kscore
RR	Relative risk
OR	Odds ratio
AUC	Area under the curve
ROC	Receiver-operating characteristics
QALY	Quality-adjusted life-years
ATC	Anatomic Therapeutic Chemical classification system for drug classification according to the World Health Organization (WHO)
TNM	Tumor, node, metastasis—stage classification of tumors according to Union Internationale contre le Cancer (UICC)
MRI	Magnetic resonance imaging
CT	Computed tomography

PREFACE

The ideas for this thesis spring from a few underlying facts that determine today's prostate cancer care. First, prostate cancer is a common and sometimes lethal disease with great impact on individuals, healthcare, and society. The widespread use of the prostate-specific antigen (PSA) test has resulted in a situation marked by frequent unorganized testing of unknown proportions—aimed at early detection in order to reduce the risk of dying from prostate cancer. From this comes over-diagnosis, whereby men who would otherwise be unaffected by a relatively benign untreated prostate cancer run the risk of having unwanted side effects from invasive diagnostic procedures and treatments.

In order to improve this situation, better tools for detection of prostate cancer are urgently needed. A number of tests have been suggested for this, but only a few are close to implementation in clinical care. Proper validation of such tools will be necessary before their widespread clinical introduction or incorporation in organized screening programs.

In addition, apart from efficient diagnosis, it is important to discuss prevention of this common cancer, which causes about one in twenty deaths in men. Although far from clinical implementation, a few common, cheap, and well-characterized medications have shown promise in preventing prostate cancer, and these require further evaluation.

In this thesis work, I have set out (1) to improve our knowledge of PSA testing behavior; (2) to validate the performance of biomarkers that are close to implementation, and (3) to investigate whether some suggested medications prevent prostate cancer.

Paper I is a registry study exploring current PSA testing. Paper II is a clinical study evaluating the performance of a genetic score when predicting prostate cancer in men with low PSA values. Paper III compares the commercially available tests Prostate Health Index (PHI) and the four-kallikrein panel in predicting prostate cancer. Paper IV is a registry study exploring prevention of prostate cancer using aspirin, statin, and antidiabetics.

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1 INTRODUCTION

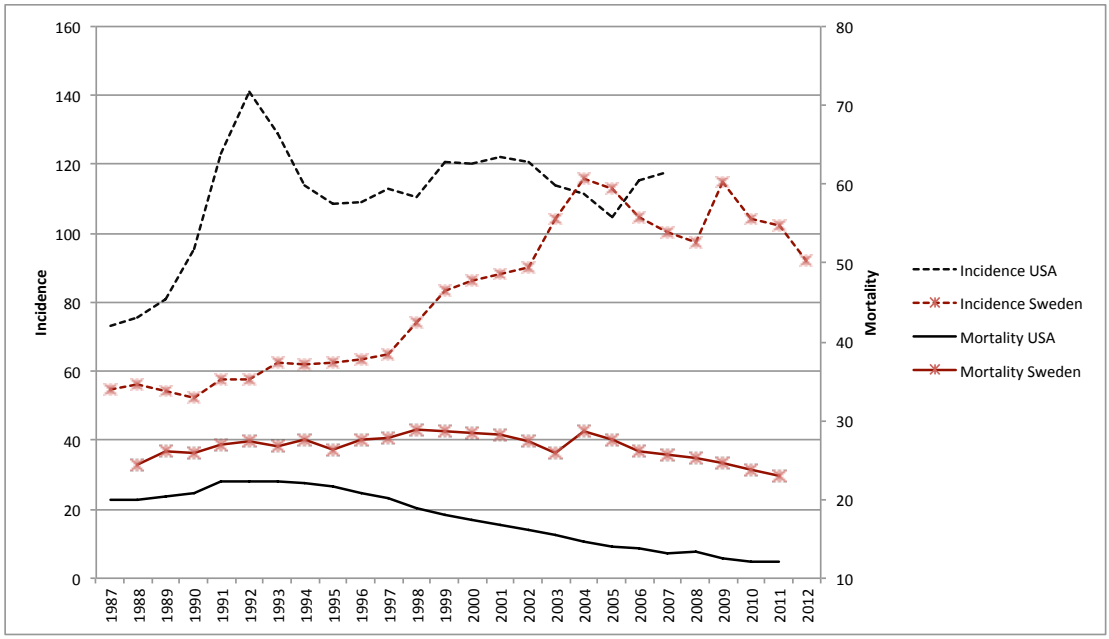
1.1 RELEVANCE OF STUDYING PROSTATE CANCER

1.1.1 Incidence and mortality

Prostate cancer is the second most common cancer in men, with an estimated 1.1 million men being diagnosed worldwide in 2012 and 70% of the cases being identified in more developed regions. The incidence varies widely between regions, with the highest incidence in North America, Australia/New Zealand, and Europe [1]. In Sweden, it is the most common cancer in males with 8,985 new cases in 2012 and a slowly decreasing incidence trend since 2009 [2]. The mortality of prostate cancer varies less, and it has been estimated that prostate cancer accounts for 6.6% of male deaths worldwide with high age-standardized mortality rates in predominantly black populations, low in Asia, and intermediate in Europe and North America [1].

After the introduction of PSA testing, the incidence of prostate cancer has increased substantially, especially in men with localized, low-grade disease. Simultaneously, mortality rates in some countries have decreased, possibly due to an increase in early treatment. On average, mortality rates have declined by approximately 3% per year 2001-2010 in the USA where PSA testing was adopted early on, and a corresponding decrease in mortality has been seen in Sweden in later years [3].

Figure 1: Age-standardized incidence and mortality rates per 100,000 people in the USA and Sweden. Incidence scale on the **left** axis and mortality on the **right**. Age-adjustment according to the world population. Data from IARC, WHO.



1.2 DIAGNOSIS AND TREATMENT

A comprehensive description of the diagnosis and treatment of prostate cancer is beyond the scope of this thesis, but can be found elsewhere [2,4]. However, it is worth making a few comments to put the work in context.

1.2.1 Diagnosis

Prostate cancer is most commonly diagnosed from prostate biopsies, a smaller fraction being diagnosed from fine-needle aspirations from the prostate, surgical specimens (e.g. TUR-P), or purely clinically. Prostate biopsies are recommended for men with an increased risk of prostate cancer, as judged by risk factors (e.g. age, family history, ethnicity, comorbidity), clinical findings (DRE, MRI, symptoms), and levels of biomarkers (e.g. tPSA, fPSA, SNPs, PHI, 4K, PCA3 etc.). In clinical practice, the level of PSA, age, and DRE findings often form the basis for estimating the risk of finding cancer on biopsy. Since the performance of the PSA test is limited (see 1.3.3.2) and the positive predictive value of a suspect palpatory finding (by DRE) is only 5–30% in patients with low PSA (< 4ng/ml) [5], additional aids are needed for decision making.

In prostate biopsy, 10–12 biopsies are most often taken under ultrasound guidance and with local anesthesia after giving antibiotic prophylaxis (commonly 750 mg ciprofloxacin before the procedure). Ultrasound gives poor visualization of prostate tumors, which is why sampling is done systematically, focusing on the peripheral zone of the prostate, which is known to harbor most cancer lesions. If indicated, additional biopsies are taken from the transitional zone. When the results show benign findings, repeat biopsies are recommended in cases with rising tPSA, suspicious DRE findings, suspicious findings on first biopsy (prostatic intraepithelial neoplasia, atypical small acinar proliferation), or persisting clinical suspicion of prostate cancer.

A variety of complications have been associated with prostate biopsies historically (hematospermia, 37%; hematuria, 14%; rectal bleeding, 2%; fever > 38.5^{o}C, 1%) [4]. The infectious complications seem to increase, and the proportion of men with post-biopsy infections due to antibiotic-resistant bacterial strains is indeed increasing. The proportion of men having a blood culture within 30 days after a prostate biopsy can be a crude approximation of the risk of severe post-biopsy infection. Using the STHLM0 cohort (see Materials 3.1), our research group has retrieved data on virtually all men in Stockholm County undergoing prostate biopsy and their blood cultures. Unpublished data (Aly M.) show an increasing proportion of men undergoing a blood culture within 30 days after biopsy and an increasing proportion of resistant strains found in those cultures. In brief, Stockholm men now appear to have more than a 2% risk of having a severe post-biopsy infection requiring blood culture, and one out of four blood cultures will show bacterial strains with signs of antibiotic resistance. The re-admission rate after prostate biopsy is 1–3% depending on the healthcare system, and the proportion suffering from a febrile urinary tract infection is approximately 3% [6].

A biopsy result is reported including the Gleason score grading and the extent of malignant findings (mm cancer; % cancer in each core). The prognosis of prostate cancer varies according to cancer stage, grade, and PSA level. Thus, the primary

staging usually includes information on DRE, biopsy findings, and PSA. Radiology (MRI, bone scan, CT scan) can be used to help estimate stage. The 2009 TNM classification for prostate cancer states that T1 corresponds to a clinically inapparent tumor; T2 is a palpable tumor confined to the prostate (T2b: more than half the prostatic lobe; T2c: bilobar nodule); T3 is a tumor extending through the prostatic capsule; and T4 is a tumor invading other surrounding structures (not including the seminal vesicles; e.g. sphincter, rectum, levator muscles). N1 and M1 refer to nodal metastasis and distant metastasis, respectively, while N0/M0 means no such metastasis and Nx/Mx means lack of information. There are several risk stratification systems, of which a modified version of the D'Amico classification is recommended in Sweden [2,7]; see Table 1.

Table 1: Classification of prostate cancer according to Swedish national guidelines [2]

Risk	Stage	Grade	PSA (ng/ml)	Additional requirements
Very low	T1–T2a	Gleason ≤ 6	< 10	< 8 mm ca. in 8–12 cores < 4 of 8–12 cores ca. PSA density < 0.15/cm^3
Low	T1–T2a	Gleason ≤ 6	< 10	
Intermediate	T2b	Gleason = 7	10–19.9	
High	T2c–T3	Gleason > 7 or extensive Gleason 4 + 3	≥ 20	

ca., cancer.

1.2.2 Prostate cancer survival

The number of men living with insignificant prostate tumors (i.e. tumors that will not affect the man if untreated) is high and age-dependent, as seen in autopsy studies and Table 2 [8].

Table 2: Prevalence of prostate cancer in a US population without prostate cancer diagnosis as found in autopsies [8].

	< 49 years	50–59 years	60–69 years	70–79 years
PCa prevalence	1 %	23 %	35 %	46 %

The risk of dying from the disease after a prostate cancer diagnosis varies widely with stage and grade. A Swedish study followed 223 men with localized prostate cancer that was not treated except for hormonal treatment after symptoms appeared [9]. At 15 years of follow-up the prostate cancer-specific survival was 89% in men with Gleason 3–6 disease, 66% in men with Gleason 7, and 28% in men with Gleason 8–10 tumors. Men with non-palpable (then T0, now classified as T1) and palpable disease (then T1–T2, now T2–T3) had similar disease-specific survival at 15 years (80%), but after 25 years of follow-up, the men with palpable disease were worse off than the men without palpable findings at diagnosis.

Converted into currently used risk grouping (using data from Rider and colleagues assessing more than 76,000 Swedish prostate cancer cases), survival when including treated men is shown in Table 3 for all ages. For a subset of men over 75 years of age, it is worth noting that even in regional metastatic disease, the cumulative risk of dying from prostate cancer was 39%—as compared to a 52% risk of dying from other causes within 10 years.

Table 3: Percent cumulative prostate cancer-specific and other-cause mortality. From Rider et al. [10]

	10-year cum. mortality (%)		15-year cum. mortality (%)	
Risk category	Prostate cancer	Other cause	Prostate cancer	Other cause
All ages				
Low risk	4.5	29	8.9	50
Intermediate risk	13	42	20	58
High risk	29	45	36	55
Regional metastatic	41	38	49	44
Distant metastatic	66	25	70	28
Age >75 years				
Low risk	7	56	10	78
Intermediate risk	15	56	20	74
High risk	29	53	33	63
Regional metastatic	39	52	42	57
Distant metastatic	63	32	64	35

1.2.3 Treatment

Treatment of prostate cancer can be summarized according to the three arms curative treatment (surgery, radiation), possible future curative treatment (active surveillance), or no curative treatment (hormonal therapy, chemotherapy, palliative radiation etc.).

For men with low risk or very low risk of prostate cancer, active surveillance with repeated PSA and DRE assessments is often the recommended choice. In intermediate-risk disease, curative treatment with radical prostatectomy or radiation therapy is recommended, provided there is an expected lifespan of more than 10 years. Results from the two trials SPCG-4 and PIVOT have been instrumental in guiding recommendations for both low-risk and intermediate risk disease [11,12]. In high-risk disease, curative treatment is also recommended in men with a shorter expected lifespan, but there is less consensus regarding which treatment modality should be preferred. The trial SPCG-7 showed that prostate cancer-specific mortality was about halved after 10 years in men with high-risk local prostate cancer treated with radiotherapy together with endocrine therapy, as compared to those treated with endocrine therapy alone (11.9% vs. 23.9%)[13]. However, a recent Swedish observational study supported the role of prostatectomy (as opposed to radiotherapy) in men with high-risk disease, and especially in young men [14]. There is a lack of trial-based results comparing prostatectomy and radiation therapy in treatment of prostate cancer. Prostatectomy or radiation therapy is seldom used for men with distant metastases, widespread regional metastases, stage T4 or PSA > 100, and hormonal treatment is the first line of treatment instead [2].

1.3 BIOMARKERS IN EARLY DETECTION

1.3.1 Classification of biomarkers

According to a classification by the US National Institutes of Health (NIH), a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmaceutical responses to a therapeutic intervention [15]. There are a number of situations where clinical decision making in cancer care can be aided by biomarkers. Six different types of biomarkers can be identified in prostate cancer, as outlined by Shariat et al. [16] and as shown in Table 4.

Table 4: Conceptual types of biomarkers

Biomarker function:	
Detection / Screening	Used for evaluation of patients with or without risk factors for PCa.
Diagnostic	Helps classical histopathological characterization in determining the presence or absence of PCa.
Prognostic	Helps prediction of outcome in patients (e.g. risk of recurrence, progression), aiding individualized management.
Predictive	Used to predict treatment effectiveness or monitor effectiveness of treatment. Can aid choice of treatment.
Surrogate endpoint	Used to substitute for a clinical endpoint and/or to measure clinical benefit. Surrogates could replace traditional endpoints, such as mortality due to disease or the recurrence or relapse of disease. Biomarkers can reduce time factors and costs for phase-I and -II clinical trials by replacing clinical endpoints.

A wide range of analyses have been proposed for prostate cancer detection. The focus of this thesis work has been on blood-based biomarkers for prostate cancer detection that are (i) in clinical use (PSA; Paper I); (ii) commercially available (PHI, 4K; Paper III), or (iii) close to clinical implementation (SNPs; Paper II). Brief descriptions of urinary markers and magnetic resonance imaging are presented, while both are interesting options in aiding biopsy decision.

1.3.2 Evaluating the usefulness of biomarkers

To be relevant for implementation, a biomarker must add value in clinical decision making. The performance of a biomarker can be described in the dimensions **discrimination** (the ability to predict prostate cancer outcome, for example) and **calibration** (if predicted risk corresponds to observed/real outcome), both of which are

necessary for clinical value. Discrimination can be described in terms of **sensitivity** (the proportion of cancer cases correctly identified by a positive test), **specificity** (the proportion of individuals with no cancer correctly identified by a negative test), **positive predictive value** (the proportion of diseased in individuals testing positive) and **receiver-operating characteristics** (ROC; plot of corresponding sensitivity/specificity by cutoff levels of the biomarker). The area under the ROC curve can be calculated as the **area under the curve** (AUC) and compared between biomarker combinations. Calibration is often illustrated by graphs correlating observed and estimated risks in a dataset.

In order to increase the possibility of drawing relevant conclusions, guidelines on how to report tumor marker studies have been presented [17]. However, drawing conclusions on the clinical value of adding a biomarker to clinical practice is more complex, and existing studies are often lacking in this (at least partially) [18]. Addressing clinical usefulness can be done through several statistical methods, e.g. decision curve analysis, net reclassification improvement, and calculation of proportion of saved biopsies vs. missed cancers.

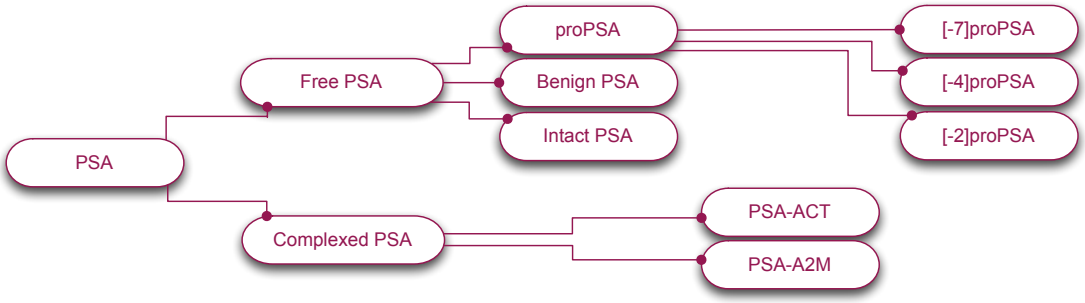
1.3.3 Current practice

1.3.3.1 Molecular forms of prostate-specific antigen (PSA)

Currently, the only widely used biomarker in prostate cancer diagnostics is measurement of the concentration of prostate-specific antigen (kallikrein 3; PSA; tPSA). It was discovered in the 1970s, but was not used in clinical medicine for another decade [19-21]. In spite of being found at low concentrations in some malignant breast, adrenal, and renal tumors, for clinical purposes it is deemed prostate-specific [22]. The kallikrein-related peptidase family member PSA is a 33-kD protein secreted by the epithelial cells of the prostate. It is found in seminal plasma at one million-fold times higher concentration than in blood serum (mg/ml amounts rather than ng/ml amounts). Conditions affecting the prostate such as prostate carcinoma, inflammation, and benign enlargement all affect serum levels of PSA, possibly through disruption of the cellular architecture [23]. In serum, most PSA is in complexed form—bound to the carrier proteins α_1-antichemotrypsin (ACT) and α_2-macroglobulin (A2M).

Five to 30% of the PSA that escapes to the blood is in unbound form (free PSA, fPSA). Free PSA exist in at least three enzymatically inactive forms: proPSA, benign PSA (bPSA), and intact PSA (iPSA), of which proPSA and benign PSA are the best characterized [24]. Whereas benign PSA is preferentially expressed in the transitional zone of the prostate and is associated with benign hyperplasia, the proPSAs are mainly expressed in the peripheral zone where malignant disease is most common [25,26]. ProPSA exists in at least three different molecular forms, where a pro-leading amino acid sequence can be truncated to different lengths, namely [-2]proPSA, [-4]proPSA, and [-7]proPSA [27]. Increasing interest has been paid to the different molecular forms of PSA as biomarkers. Total PSA and free PSA are discussed below, and the incorporation of molecular forms of free PSA and human kallikrein 2 (hK2) into predictive models are discussed in section 2.3.4 (Suggested biomarkers).

Figure 2: Outline of the molecular forms of PSA discussed.

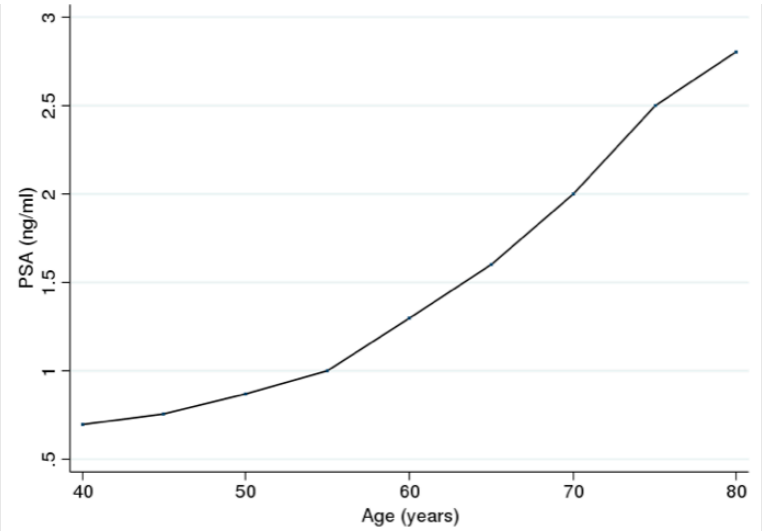


1.3.3.2 PSA as a biomarker for prostate cancer

1.3.3.2.1 Total PSA

The levels of PSA in men with no known prostate cancer increase with age [28]. This is illustrated by unpublished data from our group showing the median PSA level in 346,221 men with no known prostate cancer or no previous prostate biopsy who came for their first PSA test in Stockholm County between 2003 and 2012 (STHLM0 data, see 5.1).

Figure 3: Median PSA levels in 346,221 men in Stockholm County with no known prostate cancer and no prostate biopsy (unpublished data).



Despite the association of PSA with age and benign prostatic conditions, PSA levels are firmly associated with both the risk of significant (high-grade, high-volume) prostate cancer and the long-term risk of prostate cancer-related metastasis and death. The implementation of PSA as biomarker has revolutionized prostate cancer diagnostics, with a subsequent increase in the incidence of prostate cancer, but the test has limited specificity due to a number of biological and analytical factors [20]. The total PSA level varies approximately 20% due to temporary biological variation, thus affecting the interpretation of a single result [29]. There is additional variation between different commercial PSA assays, and care must be taken in interpreting consecutive PSA measurements that have been made according to different calibration standards (e.g. traditional vs. WHO calibration). Furthermore, common 5-α-reductase inhibitor

medication against benign prostatic hyperplasia roughly halves PSA concentrations, mostly affecting the PSA produced by benign tissue, and PSA has been suggested to have better AUC for detecting prostate cancer in men on finasteride [30].

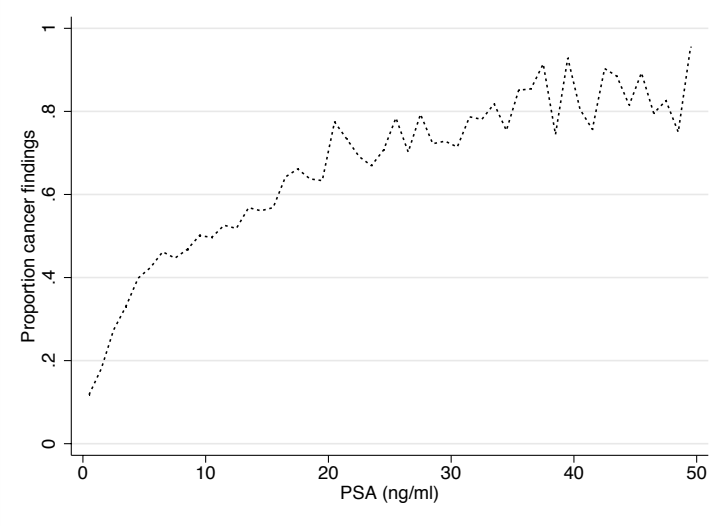
No single tPSA cutoff differentiates men with significant cancer from those with insignificant cancer (i.e. low-grade, low-volume). Similar to the presence of prostate cancer, high-grade cancer can be found in men with low total PSA levels. While high PSA levels correlate with a high risk of prostate cancer, men with moderately increased PSA levels (4–10 ng/ml) can show either malignant or benign findings. Sensitivity and specificity for PSA in finding prostate cancer have been estimated using 5578 men in the placebo-arm of the PCPT-trial (see Section 1.5.1; Age 55+; initial PSA ≤ 3ng/ml), which had a biopsy during the 7-year study. This study included invitation to end-of-study biopsies for all participants. From Table 5 it can be seen that there is no PSA cut-off with ideal properties, but rather a continuum of prostate cancer risk at all values of PSA[31]. PSA performance was slightly better in younger men (<70 years) compared to elder, possibly explained by the fact that PSA increases with age.

Table 5: Sensitivity and specificity for prostate cancer by PSA-levels. From the PCPT trial [31]

	Any prostate cancer		Gleason grade ≥7	
PSA (ng/ml)	Sensitivity	Specificity	Sensitivity	Specificity
1.1	83	39	93	37
2.1	53	73	76	67
3.1	32	87	58	82
4.1	21	94	40	90
6.1	5	99	13	98

Even in men with PSA< 4 ng/ml, about 15% will have prostate cancer and 2% will have high-grade disease [32]. This is illustrated by unpublished data from the STHLM0 cohort involving 32,348 men without prostate cancer coming for their first prostate biopsy (Figure 4).

Figure 4: Cancer proportion findings in 32,348 men in Stockholm County without prostate cancer who came for their first prostate biopsy. The men were stratified by integer PSA value and the mean proportion of cancer was calculated (unpublished data).



In contrast to this, increasing interest have been paid to baseline, mid-life measurements of PSA where low PSA concentrations are associated with a very low risk of death from prostate cancer in the long term. Using blood samples collected in 1981 from an unscreened population, Vickers and colleagues showed that a PSA level below median (1 ng/ml) by the age of 60 is associated with only a 0.5% risk of metastasis and a 0.2% risk of death from prostate cancer by the age of 85. This corresponded to 95% of deaths from prostate cancer being in men with baseline PSA above the population median at 60 years of age [33]. These results were corroborated by Ørsted and colleagues using samples collected in 1981–1983 from 4,383 Danish men. The absolute 10-year risk of prostate mortality if men had a PSA level below 1 ng/ml was < 1% in every age group, except men aged 60–64, where it was 1.4%. The risk of any clinical prostate cancer generally followed this pattern, with a 10-year absolute risk of 0.5–1.5% if PSA levels were < 1 ng/ml [34]. This has instigated suggestions that PSA tests should be recommended for men in their late 40s, while it could preclude a large number of men from further testing and the subsequent risk of over-diagnosis [35].

1.3.3.2.2 f/t PSA

PSA of men with malignant disease is less prone to proteolytic activity, and these men have a higher proportion of complexed PSA [36]. The ratio of uncomplexed (free) PSA to total PSA (f/t PSA) has been shown to increase test specificity. A prospective study of 773 men found that AUC increased from 0.53 using tPSA to 0.72 using f/t PSA in men with tPSA levels of 4–10 ng/ml and benign DRE findings. In these men, cancer was found in 56% of those with f/t PSA < 0.10 but only in 8% of those with f/t PSA > 0.25. A f/t PSA cutoff of 0.25 was therefore suggested for use in the PSA range 4–10 ng/ml where total PSA lacks adequate performance [37,38]. In 2005, a meta-analysis showed that 7–34% of biopsies could be saved at the cost of missing 2–20% of the cancers if f/t PSA cutoffs of 0.18–0.26 were introduced when deciding on biopsy in men with PSA levels of 4–10 ng/ml [39].

1.3.3.2.3 PSA dynamics and PSA density

The progress of prostate cancer is often reflected in PSA levels. Conceptually, progression of PSA can be defined in terms of PSA doubling time (PSAdt), reflecting an exponential increase in PSA, or PSA velocity (PSAv), reflecting an annual change in PSA. Historically, repeated studies have not shown any clear value of PSA velocity in the diagnosis of prostate cancer, possibly due to the biological variability of PSA, background noise (e.g. benign prostate hyperplasia), and variations in intervals between PSA measurements [40,41]. However, recent studies including a meta-analysis and a statistically advanced modeling of 219,388 men in the Kaiser Permanente health plan have shown that repeated measurements of PSA improve the detection of advanced prostate cancer as compared to a single measurement [42,43]. In summary, there is an ongoing debate on the value of PSA dynamics in aiding decisions about prostate biopsy [44,45].

PSA level depends on the size of the prostate gland, and PSA level divided by prostatic volume (PSA density, PSAD) has been associated with the risk of finding cancer [46].

An early study on 565 men identified a cutoff for deciding on biopsy of PSAD = 0.15 in men with moderately increased PSA (4–10 ng/ml) to save performing half of the biopsies (77/142) at the cost of missing about every tenth cancer (2/23) [46]. PSAD has also been associated with stage and grade, and possibly gives prognostic information [47]. Additional studies have, however, been conflicting, and the additional value of both PSA dynamics and PSA density has been questioned by the authors of the PCPT study [48].

1.3.4 Suggested biomarkers

1.3.4.1 Kallikrein-related biomarkers

Free PSA is composed of at least three different types of enzymatically inactive PSA: benign PSA (BPSA), intact PSA, and proPSA. ProPSAs are expressed in the peripheral zone and exist in several truncated forms. Through sequential steps, [-7]proPSA is truncated to PSA or [-2]proPSA. The latter has been suggested as a new prostate cancer biomarker [24,49]. Human kallikrein 2 (hK2) shows extensive amino acid sequence similarity with PSA and regulates proPSA activity. It is present in plasma in much lower concentrations than PSA, and it has been repeatedly shown to improve discrimination between men with and men without prostate cancer [50].

1.3.4.1.1 The four-kallikrein panel (4Kscore)

With the development of analytical techniques, recent attempts to increase test performance have included the development of predictive models. The group of Vickers and Lilja has explored a statistical model based on measurements of total and free PSA, intact PSA, and hK2. Based on logistic regression with linear terms (PSA and free PSA) and non-linear terms (intact PSA, hK2), the prediction model has been shown to improve discrimination between men with and without prostate cancer. Several studies have addressed the situation of biopsy decision in men with elevated tPSA. Using 2,914 men from the Rotterdam arm of the European screening study ERSPC (see section 1.4.1) where 28% of initial biopsies detected cancer, AUC for prediction of cancer in biopsy increased from 0.64 using only information on PSA and age to 0.76 with addition of information on the four-kallikrein panel. Use of the model saved 49% of the men from undergoing prostate biopsy—at the cost of missing 14% of high-grade cancers [51]. These results have been shown to be relatively stable through replication using the French and Gothenburg arm of the ERSPC [52,53].

Table 6: Overview of performance of the four-kallikrein panel aiding first biopsy decision in selected studies

				AUC for all PCa	
Reference	Year	No. of participants	PSA range	4K	Base model
Vickers et al.	2010	2,914	≥ 3	0.76	0.64
Vickers et al.	2008	740	≥ 3	0.83	0.68
Benchikh et al.	2010	262	≥ 3	0.78	0.63

Using 1,501 men coming for their second or third round of screening with an elevated PSA, the model performed slightly less efficiently, potentially reducing the number of biopsies by 36% and missing 7% of high-grade cancers (4/61). The four-kallikrein panel has also been validated for the re-biopsy setting, where patients with high risk of prostate cancer have undergone a first biopsy with benign findings, and the decision must be based on repeated biopsies. In 925 men with PSA > 3 ng/ml and a previous biopsy with benign findings, the four-kallikrein panel had superior AUC both when predicting all and high-grade (Gleason score ≥ 7) prostate cancer as compared to using tPSA and DRE alone (AUC for PCa = 0.68 vs. 0.58 and AUC for high-grade PCa = 0.87 vs. 0.76). In this cohort, with a proportion of cancer findings in the biopsies performed of 12%, the four-kallikrein panel potentially saved 54% of biopsies at a cost of missing 5% of high-grade cancers (1/19) and 34% of all cancers (37/119).

The panel has also been evaluated in 392 men undergoing radical prostatectomy for prostate cancer, where it added prognostic value when predicting aggressive disease (defined by the surgery specimen; pT3-T4, extracapsular growth, Gleason component 4–5, high tumor volume). This indicates that a number of unnecessary surgeries for insignificant tumors could possibly be avoided by implementing the panel [54].
The four-kallikrein panel is under commercialization by OPKO Health Ltd., under the name 4Kscore, where the model has been calibrated to give the risk of finding a high-grade cancer in a biopsy.

1.3.4.1.2 Prostate Health Index

Similarly, Beckman Coulter Inc. has commercialized the use of [-2]proPSA in the Prostate Health Index (PHI) algorithm, which uses the concentrations of [-2]proPSA together with tPSA and fPSA. PHI is calculated as [-2]proPSA/freePSA*√total PSA. PHI was developed with the aim of being a decision making tool in men with moderately elevated PSA with benign DRE findings [24].

One of the first study of PHI performance used 2,034 men with PSA 2,5–10 ng/ml and found an AUC of 0.77 when predicting all prostate cancer, which was superior to the use of both tPSA (AUC 0.50) and fPSA (AUC 0.68) [55]. Jansen and colleagues used 756 samples from men with PSA 2–10 ng/ml in two countries: the Rotterdam arm of ERSPC and the University of Innsbruck. They found that PHI increased AUC from 0.68 to 0.76 in predicting all cancer when compared with a base model [56]. One European five-site prospective study on 646 men with PSA levels of 2–10 ng/ml showed somewhat lower AUC (0.71 and 0.65, predicting all PCa and high-grade PCa, respectively), but still that 15% of biopsies could be avoided at the cost of missing 1% of high-grade cancers at the commonly suggested PHI cutoff of 27 [57]. Similarly, a European prospective study of 268 consecutive men with PSA 2–10 ng/ml who were undergoing initial saturation biopsies (18–24 needles) showed that PHI had superior AUC (0.76 and 0.72) compared to both tPSA and fPSA when predicting all PCa and high-grade PCa (Gleason score ≥ 7) [58]. Cutoff levels for PHI for initial biopsy decisions have been discussed by Catalona et al., using a multicenter approach with 892 men. They found a somewhat lower AUC for all prostate cancer (0.70) and argued that an option for men with a PHI of < 25 was to be followed with subsequent blood tests

rather than undergo prostate biopsy (having 11% risk of finding prostate cancer on biopsy) [59].

Table 7: Overview of PHI performance for aiding first biopsy decision in selected studies

				AUC for all PCa	
Reference	Year	No. of participants	PSA range	PHI	Base model
Le et al.	2010	2,034	2.5–10	0.77	0.50
Jansen et al	2010	756	2–10	0.76	0.68
Lazzeri et al.	2013	646	2–10	0.71	0.65
Catalona et al.	2011	892	2–10	0.70	0.53
Guazzoni et al.	2011	268	2–10	0.83	0.72

The performance of PHI in the initial and re-biopsy settings has been validated further in one European four-site prospective study involving 1,362 men with PSA levels of 1.6–8 ng/ml, showing similar results (AUC for all PCa = 0.74). For predicting outcome after radical prostatectomy (pT3 and/or Gleason score ≥ 7), PHI significantly enhanced AUC in a multivariate regression analysis, but only by 2%, and there was no evidence of clinical benefit in decision curve analysis [60].

In summary, both the four-kallikrein panel and PHI have been shown to increase test performance compared to the traditional variables tPSA, fPSA, and age when predicting prostate cancer in men coming for biopsy. All published studies are limited by the fact that men with low PSA levels are excluded from biopsy, even though we know that they can harbor significant cancer [32].

1.3.4.2 Single-nucleotide polymorphisms (SNP)

From twin studies, it is estimated that heritable factors explain 42% of the risk of having prostate cancer [61]. Indeed, the relative risk of being diagnosed with prostate cancer is 2–3 fold in brothers and sons of men with the disease, with increasing risk if the father is diagnosed at a young age [62,63]. However, more recent studies have indicated that increased diagnostic activity among the relatives of cancer patients contributes to this estimated increased risk, introducing detection bias in genetic and epidemiological studies of familial prostate cancer [64].

Attempts to decipher the heritable component of prostate cancer based on candidate gene association studies and genome-wide linkage studies in multiple-case families have suggested numerous important genes and loci. However, the inability to replicate findings suggests that prostate cancer risk is genetically complex and affected by several low-penetrance genes [65].

Single-nucleotide polymorphisms (SNPs) are single basepair-alterations that occur in the human genome when the single nucleotide (A, T, C, or G) varies at a specific location in the genome. SNPs are known to underlie differencies in how susceptible we are to diseases. They are easy to measure, and only need to be measured once, making them interesting in risk prediction models [66]. Chromosome 8q24 was the first region

to be identified in this context, and has the highest number of independent SNPs associated with prostate cancer. Since 2006, 25 prostate Genome-Wide Association Studies (GWAS) have been catalogued [67]. More than 55,000,000 SNPs have been described, and with the recent addition of 23 SNPs, 100 have been shown to be associated with the risk of prostate cancer. Known SNPs account for approximately 33% of the familial risk of prostate cancer [68].

Using data from men of different ethnicities collected in consorts, and using new whole-genome sequencing techniques, it is anticipated that future GWAS will identify even more genetic variants associated with disease. It has, however, been questioned whether these new SNPs will add value over the currently known ones[66,69]. So far, the risk-associated SNPs identified appear to be mainly associated with overall prostate cancer risk, and do not discriminate between aggressive and less aggressive disease.

1.3.4.3 Single-nucleotide polymorphisms as biomarkers

The development of robust and cheap genotyping devices has accelerated the interest in SNPs as blood-based biomarkers for cancer detection. Typically, harboring of one risk-SNP is associated with a small increase in risk (OR = 0.74–1.51), but in 2008 it was shown that a combination of five SNPs plus family history was associated with a significant cumulative effect on the risk of prostate cancer [70]. Subsequent studies have confirmed that about 10% of the male population can be identified by SNPs to have three times the median population risk of developing prostate cancer, and 1% of the population has been shown to have a 4.7-fold higher risk [68,71].

A genetic risk score can be calculated by summing the number of risk alleles (0, 1, or 2) at each of the SNPs measured, multipying it with the logarithm of that SNP's odds ratio for prostate cancer, and divide by the total number of analyzed SNPs, as used in Paper II. With this approach, our research group studied 5,241 men undergoing prostate biopsy as part of clinical practice (STHLM1). A genetic score based on 35 SNPs was constructed and estimated to potentially save 23% of biopsies at the cost of missing 8% of aggressive cancers. There was a moderate increase in AUC from 0.64 to 0.67 when the genetic score was added to a model of PSA, f/tPSA, and age. The genetic score was associated with the risk of prostate cancer (OR = 1.52; 95% CI: 1.45–1.59) [72].

The genetic score has also been evaluated in a re-biopsy situation in 1,654 men from the control arm of the REDUCE trial [73]. In this study, increasing genetic score was associated with the risk of both all and high-grade prostate cancer on re-biopsy (OR = 1.72 and 1.61, respectively). Also in this context, the increase in AUC when comparing a clinical model with and without the genetic score was modest (AUC = 0.62 vs. 0.66).

1.3.4.4 Risk calculators

Several additional statistical models incorporating biomarker levels have been suggested, while biomarkers reported individually do not perform well enough to replace PSA as the biomarker of choice in prostate cancer detection. The performance of two risk calculators in particular has been explored repeatedly.

The American PCPT was a double-blind randomized trial designed to assess chemoprevention of prostate cancer with finasteride. It included men aged more than

54 years with a PSA level ≤ 3 ng/ml and a normal rectal examination (DRE). Participants underwent prostate biopsies if PSA exceeded 4 ng/ml, and the study also included invitation to end-of-study biopsies for all participants. A risk calculator for predicting high-grade cancer (Gleason ≥ 7; PCPTHG) was developed from 5,519 men in the control arm who all underwent six-core biopsies (4.7% of whom had high-grade disease). The calculator include the risk factors PSA, DRE, African origin, age, and history of a previous biopsy [41]. A major benefit of the model is that it only contains information that is easily obtainable during outpatient visits and is readily available through websites. It does not, however, include fPSA, f/tPSA, or prostate volume—possibly weakening the results. Recently, the PCPTHG was externally validated in 10 international cohorts (25,512 biopsies; European, US, and UK cohorts). In that study, the PCPTHG discriminated better than PSA in 8 of 10 cohorts but the overall AUC was only a few percentage points better when making predictions using the risk calculator (AUC = 74.6 vs. 71.5). PCPTHG gives prediction in terms of percentage risk of high-grade cancer, but the model overestimated the risk of high-grade disease, especially in the first screening round in the Gothenburg and ERSPC trials. The calibration was better in re-screened men in the US and UK cohorts, and the model never performed worse than PSA [74].

The ERSPC risk calculators (www.prostatecancer-riskcalculator.com) consist of several calculators incorporating age, urinary symptoms, and family history (Calculator 1); PSA level (Calculator 2); and PSA in combination with DRE, prostate volume assessed by ultrasound (TRUS), and biopsy history (Calculators 3–5). The ERSPC group have argued that risk calculators should include prostatic volume since PSA level is volume-dependent, and the ERSPC calculators have outperformed the PCPT calculators in European and Canadian populations, and have performed well in a Asian population [75-77].

These risk calculators are being developed continuously, and additional biomarkers will probably be added in time. For example, a second version of the PCPT risk calculator (PCPTv2) incorporating fPSA has proven to be promising [78]. Furthermore, the urine tests PCA3 and TMPRSS2-ERG (see below) have been shown to add predictive value to the ERSPC calculator [79].

1.3.4.5 Other risk-assessment tools

Searching PUBMED for ("prostate cancer" and biomarker) yielded 19,083 hits on November 15, 2014. Despite these scientific efforts, only total and free PSA are of widespread use today—and the above-mentioned alternatives are the closest upcoming blood-based competition. However, tests based on urine have also been explored and are available in clinical practice, although to a limited extent. Furthermore, magnetic resonance imaging (MRI) has been increasingly explored for aiding biopsy decisions, targeting of lesions, and assessing patients after diagnosis of prostate cancer. To put them in context, these alternative risk-assessment tools are described briefly below.

1.3.4.5.1 Urine biomarkers

Prostate cancer gene 3 (*PCA3*) transcribes a prostate-specific mRNA that has shown promise as a diagnostic tool for prostate cancer. The PCA3 test (Progensa; Gen-Probe,

San Diego, CA) has been much studied and is in limited clinical use. It is of limited value for screening, while three strokes of prostatic massage during a DRE are needed for sufficient informative rate. When comparing ROC curves, virtually all studies have shown superiority of the PCA3 score to tPSA in predicting biopsy outcome [80,81]. However, a head-to-head comparison between tPSA and PCA3, trying to minimize attribution bias, indicated that PCA3 suffers from weaknesses similar to those with PSA, although to a lesser extent [82]. David Crawford and colleagues reported on 1,962 prospectively recruited men with PSA levels > 2.5ng/ml. As when analyzing performance of PHI and the 4K panel, they found that a number of high-grade cancers would be missed if one applied a low (10) or a high (35) PCA3 cutoff for biopsy, but 35% of biopsies could be avoided if one applied the low cutoff before deciding on biopsy [83]. The European Association of Urology states that the primary indication for PCA3 is to aid decision making on repeat biopsy following a benign first biopsy, but the cost-effectiveness remains to be seen [4]. The use of PCA3 is not supported by Swedish guidelines [2].

In prostate cancer, the transmembrane protease serine 2 (TMPRSS2) gene can be fused to an oncogene (ERG) building the gene-fusion TMPRSS2-ERG. The resulting TMPRSS2-ERG protein is present in approximately half of all prostate cancer patients. In 2006, TMPRSS2-ERG was detected in urine and showing promising specificity for prostate cancer. Although studies have shown contradictory results, several have reported an association between presence of TMPRSS2-ERG and prostate cancer stage and prognosis [84]. Neither the use of PCA3 nor the use of TMPRSS2-ERG was recommended by the Swedish Board of Medical Evaluation (SBU) in 2011 [85].

Both PCA3 and TMPRSS2-ERG have shown independent values when added to the ERSPC risk calculator including tPSA, fPSA, age, previous biopsy history, and DRE [79].

1.3.4.5.2 Magnetic Resonance Imaging (MRI) and MRI/UL-fusioned biopsies

Future aids for decision in prostate cancer must efficiently minimize the number of diagnosed tumors that would not have affected the individual if untreated (i.e. minimize over-diagnosis). One strategy for achieving this is, instead of sampling the prostate gland systematically, to only direct biopsy needles to suspected lesions—possibly detected by MRI.

Enhancement of MRI performance including functional techniques with spectroscopic imaging, dynamic contrast-infusion (DCE), and diffusion-weighted imaging (DWI) can be used. Spectroscopic imaging provides accurate metabolic information, but it is time consuming. DCE visualizes vascularity and neoangiogenesis, shows high sensitivity, but is limited by the lack of standardized protocols and analytical models. Of the functional MRI techniques, DWI is the most readily used, relying on the fact that the freedom of movement of water molecules (the diffusion coefficient) is restricted in cancer lesions due to reduced extracellular space [86].

MRI images can be used with biopsy needle placement when in the MRI tube (“in-bore” biopsies) or by fusing the images to ultrasound (UL/MRI fusion), enabling tumor-guided biopsies using ultrasound in a regular outpatient setting. In-bore biopsies

are accurate, but they are time and cost consuming [87]. UL/MRI fusioned biopsies are intuitively attractive and have been explored prospectively by Pokorny and colleagues. These workers performed both fusioned biopsies against MRI-detected lesions and traditional systematic biopsies in 223 asymptomatic men. They found that 29% of cancers were not located in the peripheral zone, which is traditionally the only biopsied part of the prostate. Sensitivity and specificity of the fusion biopsies were superior to those of the systematic biopsies, and they concluded that compared to traditional biopsies fusion biopsies reduce the detection of low-risk cancers (n = 43 vs. 97), reduce the need for biopsy (n = 142 vs. 223), and improve the overall detection of intermediate/high-risk prostate cancer (n = 93 vs. 79) [88] (Table 8).

Table 8: Histology of systematic biopsies and UL/MRI fusioned biopsies in 223 consecutive asymptomatic men. Combined histology was determined by the highest-risk tumor by either biopsy method. From Pokorny et al. [88]

Histology result	Systematic biopsies	Fusion biopsies	Combined findings
Benign	97	43	81
Low-grade cancer	47	6	34
Intermediate/high-risk cancer	79	93	108
Total	223	142	223

1.4 SCREENING FOR PROSTATE CANCER

The underlying concept of screening is that early detection of risk factors or early disease is beneficial for clinical or public health outcome. As early as in 1968, Wilson and Jungner suggested a number of criteria to be met before introducing a screening program in a population [89]. Since then, criteria have been refined, but can still be summarized in the following four categories: (A) **knowledge of the disease** (importance for health, natural history, time from sub-clinical disease to overt disease, benefits of early detection); (B) **the test** (validity, reliability, cost); (C) **the diagnosis and treatment** (availability, cost-effectiveness, sustainability, harm/benefit balance); and (D) **the screening program** (who should be treated as a patient, health economy).

Several studies have addressed the effects of PSA-based screening for prostate cancer, of which the randomized trials ERSPC and the PLCO have been the most prominent and have sparked a vivid scientific debate. ERSPC included 182,160 men aged 50–74 years from six European centers, randomly assigned to either a group that was offered PSA screening or a control group that did not receive any PSA screening. Men in the screening group underwent 2–5 PSA screening rounds at 4-year intervals (10 rounds and 2-year intervals in the Swedish arm). A cutoff of 3 ng/ml was used as indication for biopsy. The ERSPC found a 21% lower relative risk of prostate cancer mortality after 13 years in PSA-screened men (rate ratio = 0.79; 95% CI: 0.69–0.91) [90]. This corresponds to 781 men having to be invited to screening and 27 men having to be diagnosed for each prostate cancer death avoided. The Gothenburg randomized trial was initiated in Sweden and involved 20,000 men randomized to biannual PSA screening or to a control group not invited for testing. Background PSA testing in Sweden was very low, and after 14 years of follow-up the cumulative prostate cancer

incidence was 12.7% in the screening group and 8.2% in the control group. The mortality from prostate cancer was almost halved (rate ratio = 0.56; 95% CI: 0.39–0.82) in screened men after 14 years. Overall, 293 men needed to be screened and 12 needed to be diagnosed to prevent 1 prostate cancer death at 14 years. The Gothenburg trial reported most of its patients to the ERSPC [91].

The American PLCO trial was instigated approximately simultaneously with ERSPC. It included 76,685 men aged 55–74, and men in the screening arm underwent annual PSA testing and rectal palpation. American men used PSA testing earlier than European men, and at least 40% of men in the PLCO control arm had their PSA taken during the study. In addition, almost a third of the men in each arm had had a PSA test before the trial (pre-screening). From this, it might be expected that the power of the trial would be reduced. It has therefore been argued that the PLCO is underpowered and not a screening trial, but rather a comparison between different approaches to screening (i.e. opportunistic vs. systematic) [92,93]. The PLCO showed no mortality benefit with PSA screening after 13 years, finding no difference in disease-specific mortality rates between the groups (RR = 1.09; 95% CI: 0.87–1.36). The incidence of prostate cancer was generally higher in the screening arm than in the control group (RR = 1.12; 95% CI: 1.07–1.17) [94].

Prostate cancer screening did not significantly decrease prostate cancer-specific mortality in a combined meta-analysis of five trials done by the Cochrane Library [95].

A major drawback of PSA screening is the increased prostate cancer detection in men with disease that would not have affected their lives if not diagnosed, resulting in high risk of over-treatment. Using microsimulation models, it has been demonstrated that the benefit of PSA screening in ERSPC was diminished by loss of quality-adjusted life-years (QALY), owing to long-term effects caused by side effects of diagnosis and treatment [96].

In order to concentrate screening on those who benefit, Carlsson and colleagues recently studied stratified screening. They compared risks of prostate cancer metastasis and death in two PSA-stratified cohorts of men aged approximately 60 years, the first from the screened arm in the Gothenburg screening trial (screening) and the second from the historical Malmö Intervention trial (control). They reported that for men aged 60 years with an initial PSA level of ≥ 2 ng/ml, only 23 needed to be screened and six needed to be diagnosed to prevent one death after 15 years. In contrast, men with PSA levels < 1 ng/ml had a very low risk of metastasis and death [97].

In summary, many authors have argued that the time for population-based screening using PSA levels has not arrived [90]. Instead, following five golden rules for transforming PSA screening in order to minimize over-treatment has recently been suggested: (1) **Obtain consent from the participants**; (2) **Do not screen men who will not benefit**; (3) **Do not perform biopsy without a compelling reason**; (4) **Do not treat low-risk disease**; and (5) **If treating, do so at high-volume centers** [98].

In line with this, today most organizations addressing this issue do not recommend screening with PSA, but rather offering early diagnostics and treatment to well-informed men. The American Urological Association recommends shared decision making and— if agreed—PSA testing at intervals of two or more years in men aged 55–69 years, possibly also in younger men with high risk due to heredity or ethnicity. A recent Best Practice Statement update suggested that the age for obtaining a baseline PSA be lowered to 40 years and the single PSA threshold for biopsy should be replaced by a decision based on tPSA, DRE, f/tPSA, age, family history, previous biopsy history, and comorbidities [99].

The National Comprehensive Cancer Network (NNCN) recently uniformly recommended that baseline PSA testing should be recommended for healthy, well-informed men aged 50–70 years, with a lower grade recommendation also for men aged 45–49 years. Testing in men > 70 years should be performed with caution, and only in very healthy men with little comorbidity—since these men often harbor prostate cancer of insignificant importance. PSA testing should be repeated at 1- or 2-year intervals, except for men aged 45–49 with PSA < 1 ng/ml where further testing can be deferred until the age of 50 [93].

These recommendations followed on from the much discussed recommendation of the United States Preventive Services Task Force (USPSTF) in 2012 against PSA screening. They argued that there is convincing evidence that PSA-based screening programs result in the detection of many cases of asymptomatic prostate cancer, and that many men who have asymptomatic cancer detected by PSA screening have a tumor that either will not progress or will progress so slowly that it would have remained asymptomatic in the man's lifetime (i.e. over-diagnosis). Because of the inability to identify tumors that will remain indolent from those destined to be lethal, many men are being subjected to the harms of treatment for prostate cancer that will never become symptomatic (i.e. over-treatment). Together with the Canadian Task Force on Preventive Health Care (CTFPHC), they argued that the benefit of a small decrease in prostate cancer mortality seen in the ERSPC trial does not compensate for the substantial risks of diagnosis-related psychological harm, biopsy complications, post-treatment side effects[100,101].

In 2013, the European Association of Urology published four statements, including that PSA screening reduces PCa-related mortality, that baseline serum prostate-specific antigen level should be obtained at 40–45 years of age, and that intervals for PSA testing should be 2–8 years depending on the results of previous PSA testing [102]. In the most recent EAU guidelines on prostate cancer screening and early diagnosis, men are recommended to undergo biopsy after shared decision making, and using a threshold PSA for biopsy of 2–3 ng/ml [4]. The Swedish guidelines from 2013 recommend against PSA screening, but promote organized PSA testing in men aged 50–70 years after informed decision making [2].

1.5 CHEMOPREVENTION

Being a common disease causing a relatively high number of deaths, it is relevant to study possible chemoprevention in prostate cancer. To date, no pharmaceutical drug has been evaluated with sufficient results to be recommended for patients. However, a few medications have been suggested and have shown different levels of promise. Alfa-reductase inhibitors have been studied in trials, but there have only been observational studies on aspirin, statins, and metformin.

1.5.1 Alfa-reductase inhibitors

5-alfa-reductase (5-AR) converts testosterone to di-hydrotestosterone, and can be inhibited by 5-alfa-reductase inhibitors (5-ARI; e.g. dutasteride and finasteride). Long-term observations of individuals with inherited 5-AR defiency have shown a reduced growth of the prostate and diminished risk of prostate cancer, inspiring studies on prevention of prostate cancer with 5-ARIs [103]. Two large randomized trials have been performed addressing the use of alfa-reductase inhibitors in preventing prostate cancer. The PCPT trial was designed to assess the ability of finasteride to reduce detection of prostate cancer in men at low risk of the disease. Finasteride reduced the risk of prostate cancer by 25%, but men in the treatment arm showed a slightly higher risk of high-grade cancer (6.4% vs. 5.1%). Sexual side effects were more common and urinary symptoms were less common in the treatment group than in the placebo group [104].

The REDUCE trial included men with PSA levels of 2.5–10 ng/ml. The trial showed that dutasteride reduced the risk of prostate cancer by 23%, corresponding to an absolute risk reduction of 5%. However, there was no risk reduction for Gleason 7+ tumors. In addition, there were significantly more cardiac events in the dutasteride group (0.7% vs. 0.4%) [105].

Table 9: Overview of the PCPT and REDUCE trials

	PCPT (finasteride) [104]	**REDUCE** (dutasteride) [105]
n	18,882	8,231
Age; PSA	≥ 55 years; ≤ 3 ng/ml	50–75 years; 2.5–10 ng/ml
Follow-up	7 years	4 years
All PCa	18.4% vs. 24.4%; p < 0.001	19.9% vs. 25.1%; p < 0.001
High-grade PCa (Gleason 7+)	6.4% vs. 5.1%; p = 0.005	6.7% vs. 6.8%
NNT to prevent one cancer	17	20

PCa , prostate cancer; NNT, number needed to treat.

A lively debate followed from the results of PCPT and REDUCE, especially about the risk of high-grade tumors in men treated with 5-ARI. Neither finasteride nor dutasteride has been approved by the FDA for prevention of prostate cancer. However, 18-year follow-up of the PCPT study showed no difference in overall survival or prostate cancer-specific survival between men treated with placebo or finasteride [106]. This

suggests that 5-ARI reduces the risk of low-grade prostate cancer without affecting the long-term risk of death from prostate cancer.

1.5.2 Aspirin

There is laboratory evidence to suggest that chronic inflammation plays an important role in the etiology of cancer. Several studies have suggested a protective effect from anti-inflammatory drugs such as aspirin on the risk of developing various forms of cancer, including prostate cancer [107]. One meta-analysis by Bosetti and colleagues found a 10% reduced risk of prostate cancer in regular aspirin users, based on data from nine case-control and 15 cohort studies, although there was significant heterogeneity in the risk estimates and no relationship between risk reduction and frequency, dose, or duration of use [108]. Another, more recent, meta-analysis showed similar results with a significant inverse association between aspirin and the risk of both overall and high-risk prostate cancer (OR = 0.92 and 0.81). In this analysis, there was also a significant association with prostate cancer mortality (OR = 0.86) [109]. At the same time, Shebl and colleagues used data from 29,450 men in the PLCO trial and showed a protective effect of daily aspirin on prostate cancer risk (HR = 0.92; 95% CI: 0.85–0.99) [110]. However, in 2014 Veitonmäki used 78,615 men in the Finnish screening trial and reported an increased risk of prostate cancer in NSAID users (HR = 1.45; 95% CI: 1.33–1.59), but no significant association between prostate cancer and use of aspirin [111]. Murad et al. investigated associations between NSAID and aspirin use and prostate cancer risk in the ProtecT trial and found no protective effect, but they also reported an association between aspirin used and reduced serum PSA in controls [112].

Algotar investigated 140 men with prostate cancer, showing lower baseline PSA in non-users of aspirin (PSA 5.2 ng/ml vs. 7.6 ng/ml) [113]. Fowke and colleagues investigated 1,277 men scheduled for prostate biopsy and found lower PSA levels in aspirin users (7.3 ng/ml vs. 12.7 ng/ml) [114] . While biopsy decisions are based on PSA levels, anything that lowers PSA levels might reduce the incidence of prostate cancer while some men are withdrawn from biopsy. Addressing this potential detection bias in observational studies is important.

1.5.3 Statins

Also being a very commonly prescribed medication, statins have been proposed as possible chemopreventive agents. In a matched case-control study using data on 387 men who died from prostate cancer, Marcella and colleagues found a substantial degree of protection against prostate cancer death in statin users (OR = 0.49; 0.34–0.70) [115]. Tan et al. used a retrospective cohort design on 4,204 men undergoing prostate biopsy. They found a reduced risk of prostate cancer (RR = 0.86; 95% CI: 0.75–0.97) and also less frequent high-grade prostate cancer [116]. Using the Veterans Affairs Health Care System, Farwell identified 55,875 American men on statin or hypertensive medication. Compared to men on antihypertensive medication, statin users were 31% less likely to be diagnosed with prostate cancers (HR = 0.69; 95% CI: 0.52–0.90) [117]. A Finnish cohort study of 23,320 men participating in a screening trial demonstrated a dose-

dependent reduced prostate cancer incidence among statin users as compared to non-users (HR = 0.75; 95% CI: 0.63–0.89). The inverse association was strongest for low-grade and early-stage tumors [118]. It is especially interesting that this study—while using data from the Finnish PSA-screening study—described this effect and at the same time corrected for age-adjusted PSA levels.

The effect on PSA from statin use is quite well established. A biological causality has been proposed, and cohort studies have shown lower PSA levels in statin users—as shown in a review by Mener [119]. He demonstrated this in a cohort of 962 patients, with 8% lower PSA levels among users [120], which is congruent with the findings of Murtola's Finnish group [118].

1.5.4 Metformin

Metformin is a biguanide with antihyperglycemic effects. It acts through reduction of liver production of glucose and by increasing the peripheral sensitivity to insulin. Common side effects include changes in taste and a diversity of gastrointestinal symptoms that most often diminish gradually.

An increasing number of studies are investigating a possible association between metformin and the risk of cancer, although the results are contradictory. In 2012, a meta-analysis covering 37 studies (7 on prostate cancer) showed risk reduction for liver (78%), pancreatic (46%), colorectal (23%), and breast cancer (6%). However, no significant association between metformin and risk of prostate cancer was seen [121]. A later meta-analysis on mortality risk after cancer diagnosis in men with or without metformin has come to similar conclusions [122].

In 2013, David Margel and colleagues published two articles on the association between metformin treatment and the risk of prostate cancer, its grade, and disease-specific mortality [123,124]. The first was a registry-based retrospective nested case-control study involving 5,306 diabetics on metformin and 26,530 diabetic controls. They found no significant association between metformin and any prostate cancer (OR = 1.03; 95% CI: 0.96–1.1) or high-grade prostate cancer (OR = 1.13; 95% CI: 0.96–1.32) [124]. Using the same registry-based retrospective cohort, they published mortality data a few months later using a cohort study design with 3,837 diabetic men who developed prostate cancer. The cumulative duration of metformin medication was associated with a reduced risk of prostate cancer-specific death in a dose-dependent fashion (HR = 0.76; 95% CI: 0.64–0.89) for every six months of additional metformin use, and the study sparked interest in a future randomized trial [123]. This finding was, however, questioned by Marjoleen Zanders in an article suggesting that confounding by indication might explain the 24% reduction in mortality per six months of metformin used—per se not entirely biologically plausible [125]. The term "confounding by indication" is usually used to denote a particular form of confounding in studies of medications where the apparent effect of a medication is actually a result of the condition for which it is prescribed.

2 AIMS

2.1 OVERALL AIMS

The overall aims of this thesis were to

1. explore the current practice in prostate cancer testing
2. evaluate proposed biomarkers for early detection of prostate cancer
3. investigate chemoprevention of prostate cancer.

2.2 SPECIFIC RESEARCH QUESTIONS

During the project, several specific scientific questions were raised. Among these, this thesis was based on the following questions.

2.2.1 Paper I

How prevalent is testing and retesting of PSA?

2.2.2 Paper II

Is a genetic score based on single-nucleotide polymorphisms informative regarding the risk of prostate cancer in men with low PSA?

2.2.3 Paper III

Are the commercially available models PHI and the four-kallikrein panel comparable in aiding biopsy decisions?

2.2.4 Paper IV

Do commonly used medications affect PSA and the risk of prostate cancer on biopsy?

3 MATERIALS

This thesis work was performed using data from two main sources that were organized during the project period. Paper I and Paper IV used data from the STHLM0 cohort and Paper III used data from the STHLM2 cohort. Paper II was a clinical study with subjects invited from STHLM2.

3.1 STHLM0

3.1.1 Base cohort

The STHLM0 cohort covers all men in Stockholm County who have had a PSA test since 2003. It is continuously updated with data from all known laboratories in the area that perform PSA or biopsy analyses (Karolinska University Laboratories, Aleris, and Unilabs), involving up to 422,000 participants up to September 2014. Data on all analyzed PSA samples are continuously acquired together with SNOMED-coded biopsy results on each individual prostate biopsy session involving the cohort participants. The place of residence is determined through linkage with Statistics Sweden. The base cohort is then intermittently updated against the registers listed below, which are held by regional cancer centers, the Swedish National Board of Health and Welfare, and Statistics Sweden.

STHLM0 is kept at the Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, as an Oracle-based database, where researchers (after agreement) can access anonymized data for analysis.

3.1.2 Registries held at the Swedish Board of Health and Welfare

3.1.2.1 The Swedish Cancer Register

The Cancer Register was started in 1958 and is the oldest health register in Sweden. It is held by the Swedish Board of Health and Welfare and reporting is mandatory by law. All physicians must report all new cases of cancer, and separate registrations are done by pathologists and cytologists with additional data. Thus, two separate departments identify most cancer cases. The register covers more than 96% of the approximately 60,000 malignant tumors diagnosed annually in Sweden. It includes information on personal identity, sex, place of residence, diagnostic unit, ICD-coded tumor localization, date of diagnosis, and tumor stage at diagnosis as described by TNM status. Data are collected by the six regional cancer centers and quality assessment of the data is performed both by the cancer centers and the Board of Health and Welfare [127]

3.1.2.2 The Swedish Cause of Death Register

Since 1960, the causes of death of all Swedish citizens have been registered in the Swedish Cause of Death Register, which is updated on an annual basis. Since 1997, it has also included persons for whom the death certificate, but not the cause-of-death form, has been registered. The register includes variables on age at death, date of death, place of death, sex, and the immediate and contributory causes of death coded

according to the ICD (International Classification of Diseases). The register includes data on the cause of death in 98% of all deaths, and agreement between the registry and peer-reviewed journals has been reported to be 86% in prostate cancer patients, indicating a fairly high degree of validity [128].

3.1.2.3 The Swedish Prescription Register

All prescription medications that are dispensed in Sweden are reported to the National Prescription Register, corresponding to 100 million prescriptions annually. The register is updated monthly and includes variables on patient ID, prescriber, place of prescription and expedition, the expedited medication code according to the ATC (Anatomical Therapeutic Chemical Classification System), and cost. It is increasingly used for scientific purposes, since the personal identification number of the patient has been included in the register from 2005. Data are collected by a collaboration service run by the pharmacies (Apotekens Service) and reported to the registry, which is held by the National Board of Health and Welfare. The coverage exceeds 99% of all prescriptions dispensed, indicating high validity. Despite this, the register must be used with caution for certain medicines, and medications bought over the counter are not registered.

3.1.3 Registries held at Statistics Sweden (Statistiska Centralbyrån)

3.1.3.1 Population and socioeconomic data

Statistics Sweden (Statistiska Centralbyrån; SCB) holds a wide variety of data on Swedish citizens in the **population registers** (Registret över totalbefolkningen, Registret över inkomster och taxeringar (income register), Förmögenhetsregistret, Registret över Kapitalvinster och -förluster, Folk- och Bostadsräkningen) and **activity registers** (Befolkningens utbildning, Elevregistret, Komvuxregistret, Universitetens register, Lönesummestatistiken, Yrkesregistret). Furthermore, data are collected in surveys on household economy, salary structures, etc.

Data on birth, death, and migration are kept on each Swedish citizen and are reported by the Tax Agency (Skatteverket) to the Total Population Register (Registret över totalbefolkningen).

The population censuses (Folk- och Bostadsräkningen) have been performed several times, but most recently in 1990, which is why such data are becoming increasingly outdated. Instead, the register on education of the population (Befolkningens utbildning) and the economic registers provide important socioeconomic information. There is also important information in the Employment Register (Yrkesregistret), but this is more difficult to use while different professions are coded with low quality.

The register on the population's education is updated annually, and data on education accomplished is reported to the registry from all Swedish schools. Highest education achieved is coded according to SUN-2000 (Svensk UtbildningsNomenklatur) and entered together with personal identification number, place of education, and year of education. The SUN coding is stratified in seven levels, which are readily and often transcribed to three education strata representing 0–9, 10–12, and > 12 years of education (school, high-school, and further or higher education).

3.1.4 The National Prostate Cancer Register (NPCR)

In the 1990s, all six Swedish regions established regional prostate cancer registries. In 1998, the NPCR was created from these and is now held by a collaboration between the Regional Cancer Centers in Sweden. It includes diagnostic unit, date of diagnosis, and tumor stage according to the TNM classification system. It also includes extensive clinical information on Gleason grade, PSA level at diagnosis, and primary treatment within six months of diagnosis. Reporting is coordinated with reporting to the Swedish Cancer Register, and involves three different forms: one diagnostic form, one form on primary treatment, and a separate form on radiotherapy. Reporting is internet-based and voluntary. Of 103,047 men registered in the Swedish Cancer Register between 1998 and 2009, 100,849 were registered in the NPCR, illustrating the very high capture rate [129,130].

3.2 STHLM2

3.2.1 Description

Our research group collected the STHLM 2 cohort between November 2010 and September 2012. At 60 test laboratories in Stockholm County, all men coming for PSA testing were invited to give four extra tubes of blood, to give a urine sample, to have their blood pressure measured, and then to answer a questionnaire—building a population-based cohort. Altogether, 24,642 men were included during the 22-month study period. For the base cohort, no age restriction was used and participants could enter the cohort repeatedly if they came for repeat PSA testing. Historical PSA test data, biopsy records, clinical records, socioeconomic data, and prescription data were retrieved from the STHLM0 database.

3.2.2 Collection and biobanking

Apart from the blood drawn for standard PSA analysis, the study used separate study laboratory referrals for blood collection. Whole blood for plasma and DNA was collected in ethylenediaminetetraacetic acid (EDTA) tubes without gel. The tubes were transported to KI Biobank within 24 hours. Centrifugation and aliquoting of plasma (225 µl) was performed in an automated system and the samples were stored at −80°C. DNA was extracted using magnetic bead separation. Participants entered questionnaire data through a website or using mailed paper forms if preferred.

4 METHODS

4.1 PAPER I

For Paper I, the study population was defined as all males living in Stockholm County on May 28, 2012 (n = 1,034,129), as verified using data from the population register. Data on PSA tests, prostate biopsies, and cancer diagnoses in these men were retrieved from the STHLM0 cohort described above. Aggregated data on the study population were retrieved from Statistics Sweden. Historical population estimates were calculated for single years by multiplying the 2011 population by the probability of not immigrating to Stockholm in the intervening years. The calculation of historical population sizes enabled the calculation of test prevalence throughout the study period (2003–2011). In papers I–IV, STATA versions11–13 (StataCorp LP, College Station, TX, USA) were used for the main parts of the statistical analysis.

4.1.1 Prevalence calculations

Duration-specific test prevalence was calculated as the number of men having a PSA test in Stockholm preceding a point in time divided by the population at that point in time. Prevalence was stratified in 10-year age groups excluding men outside the age range 40–89 years.

4.1.2 Survival analysis

Pattern of PSA retesting was illustrated using survival analysis. Cumulative incidence of a second PSA test was calculated using time since the first test as underlying timescale. End of follow-up in the survival analysis was date of retesting, date of prostate cancer diagnosis, or December 31, 2011, whichever came first. We excluded men less than 40 or more than 89 years of age together with men who had previously undergone prostate biopsy and men with prostate cancer.

4.2 PAPER II

The study population of Paper II was men in STHLM2, aged 50–69 years, coming for PSA testing without previous prostate biopsy or prostate cancer and with a PSA level of 1–3 ng/ml. We identified 2,696 men from the STHLM2 cohort from whom we could make a stratified random selection based on a genetic score.

Men were stratified and invited according to risk deciles, with the lowest risk decile corresponding to low risk, the highest decile corresponding to high risk, and the remaining deciles defining the intermediate risk group. The clinicians involved in the study were blinded regarding risk score, until data analysis.

Considering that prostate biopsy can have medical side effects, we restricted the study size to meet the requirements stipulated by power calculations, and we over-sampled participants from the highest and lowest risk groups. Altogether, 860 men were invited by letter and 172 of them underwent prostate biopsy and complete data analysis (n =

50; n = 79; and n = 43 from the first, second to ninth, and tenth risk deciles, respectively); 668 men did not answer the invitation.

4.2.1 Calculation of genetic risk score

Blood plasma from the entire study cohort (n = 2,696) was genotyped for 50 single-nucleotide polymorphisms (SNPs) identified as being associated with prostate cancer risk. Genotyping was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry based on allele-specific primer extension with iPLEX chemistry (SEQUENOM 96 Inc., San Diego, CA, USA).

For each man, we created a genetic score by summing the number of risk alleles (0, 1, or 2) at each SNP and multiplying by the logarithm of that SNP's reported odds ratio, then dividing by that man's total number of called SNPs. Men were then assigned a risk decile according to genetic score rank.

4.2.2 Sampling, biopsy procedure, and specimen evaluation

In total, 192 biopsy occasions were arranged for invitees to book through an internet-based booking system or by telephone. Biopsies were performed by myself or Dr. Markus Aly between May and December, 2012, following clinical guidelines. Between 10 and 12 ultrasound-guided biopsies were taken from the peripheral zone.

Each specimen was fixed separately, paraffin-embedded, and cut at 4 µm. Professor Lars Egevad, who was blinded regarding the PSA level and risk score of the participant, reviewed biopsies.

4.2.3 Logistic regression analysis

Associations between prostate cancer on biopsy and evaluated risk factors were explored in univariate and multivariate logistic regression analyses, using STATA software. The multivariable model included genetic risk score, total PSA, PSA ratio, age, prostate volume, digital rectal examination (DRE) findings, and family history.

4.2.4 Net reclassification improvement (NRI) analysis

Two multivariate logistic regression models including and excluding the genetic score were compared using net reclassification improvement (NRI). The NRI considers a situation where a given event is predicted by two models, in this case one model using tradition variables (total PSA, PSA ratio, family history, prostate volume, DRE) and one model using these variables plus the genetic score.

The predicted probabilities of changing classification (cancer/no cancer) when changing between the models are calculated and NRI is then calculated as:

$$NRI = [P(up|cancer) - P(down|cancer)] - [P(up|nocancer) - P(down|nocancer)]$$

Where *up/down* is upward/downward classification (from no cancer to cancer and vice versa) using the new model and *cancer/nocancer* is the true value the model tries to predict. Followingly a suggested model should have a positive NRI to be of clinical value and the NRI ranges from −2 to 2 [131].

4.3 PAPER III

Paper III compared the performance of three models (base model, PHI, 4K) predicting all and high-grade prostate cancer. It was a prospectively collected, observational study including men with PSA test taken before a prostate biopsy resulting in cancer diagnosis (cases) or benign findings (controls). We selected all new prostate cancer cases in the STHLM2 cohort and all men having a biopsy with benign findings after inclusion in STHLM2. For the main analysis, we only included previously unbiopsied men with PSA levels of 3–15 ng/ml, while men with PSA < 3 ng/ml do not routinely come for biopsy and men with PSA > 15 ng/ml would often be biopsied regardless of the result of a new biomarker test. Clinical data were drawn from the STHLM0 database.

Biopsies were performed by clinicians in Stockholm according to clinical practice, including information on PSA levels, DRE findings, prostate volume, and family history.

Plasma aliquots from the STHLM2 cohort were sent for analysis to the laboratories listed below:

Biomarker	Laboratory	Analysis equipment
Total PSA, free PSA	Karolinska Universitetslaboratoriet	Roche Modular E170
[−2]proPSA	Karolinska Universitetslaboratoriet	UniCel Dxl 800 (Beckman Coulter)
hK2, intact PSA	Wallenberg research laboratory, Skåne University Hospital	Research Assay[132]

4.3.1 Evaluated biomarker models

A base model with age and total PSA was modeled using logistic regression. The PHI score was calculated as [−2]proPSA/freePSA*√total PSA [24]. The four-kallikrein model was calculated by Andrew Vickers using a previously described method incorporating restricted cubic splines [133]. These models were then used to predict the risk of finding prostate cancer, providing entirely external validation.

4.3.2 Receiver-operating characteristics (ROC)

Discrimination was assessed using receiver-operating characteristic (ROC) curves and summarized using the area under the ROC curve (AUC). Briefly, the ROC curve illustrates the performance of a model with a binary endpoint (e.g. cancer/no cancer) over a set of threshold values (e.g. different levels of a biomarker). It is created by plotting the sensitivity (the probability of a positive test given that the patient is ill)

against the specificity (the probability of a negative test given that the patient is well) of the biomarker at various thresholds.

4.3.3 Decision curve analysis

Decision curve analysis was introduced by Vickers and Elkin in 2006 as a tool to evaluate whether new models improve clinical management of patients [134]. Briefly, decision curve analysis graphically illustrates the net benefit obtained from using the predictive models in a patient. This is done by assuming that there is a threshold probability of having prostate cancer at which a patient decides to undergo biopsy and assuming this is informative of how the patient weighs the relative harms of a false-positive or false-negative prediction. This relationship is used to derive the net benefit of the model across different threshold probabilities. Plotting of net benefit against the threshold probabilities gives the "decision curve".

4.3.4 Calculation of the percentage of biopsies avoided

Prostate biopsies cause harm in terms of physical pain, mental distress, risk of diagnosing cancer that would otherwise not have affected the person's life (over-diagnosis), and increasing infectious complications with antibiotic-resistant bacteria. Thus, it is essential that biomarker models that are introduced should increase test specificity, with maintained sensitivity for aggressive tumors. In short, it is of utmost importance to avoid unnecessary biopsies.

The percentage of potentially avoided biopsies using a prediction model can be calculated as the number of avoided biopsies by different threshold levels of the biomarker. The calculation can be written $(TP_1 + FP_1 - TP_2 - FP_2) / (TP_1 + FP_1)$, where TP and FP denote counts of true and false positives in the two models being compared.

The number of missed cancers is equally important, and can be calculated as the false negatives.

4.4 PAPER IV

Paper IV was a retrospective registry-based cohort study addressing the association between aspirin, statin, and anti-diabetic medication on the one hand and PSA levels and the risk of finding prostate cancer in a biopsy specimen on the other. The underlying study population was drawn from the STHLM0 cohort, representing virtually all PSA-tested men in Stockholm. Since prescription data were available from 2005, we included men undergoing a PSA test between 2007 and 2012, allowing a run-in period and at least 12 months for verification of diagnosis.

Exposure to medication was assessed through the prescription register, and drug use was defined as any prescription dispensed within two years before biopsy. Cumulative dose was calculated as quartiles of total dose in the two years preceding biopsy.
The endpoints were (i) level of first PSA in men without prior prostate biopsy or prostate cancer and (ii) the risk of prostate cancer and high-grade prostate cancer in a man's first prostate biopsy. Regression analysis was adjusted for education level

(achieved from Statistics Sweden), PSA levels, and Charlson Index (calculated from the National Patient Register).

4.4.1 Calculation of differences in PSA level

The distribution of tPSA in a population is left skewed due to the fact that men with advanced prostate cancer often show with very high PSA levels (thousands). Geometric means are a way of dealing with this, and can be calculated as $g = \sqrt[n]{x_1 x_2 \ldots x_n}$, where x denotes the individual PSA measurements. To illustrate differences in PSA levels, we fitted a linear regression on natural log-transformed PSA levels with adjustment for age, comorbidity, and medications. We then calculated the anti-logs, giving the difference in PSA levels between groups in percentage points.

4.4.2 Association between medication and biopsy result

The association between a medication and the risk of finding prostate cancer in a man's first biopsy was addressed using first univariate and then multivariate logistic regression adjusted for age, log-transformed tPSA, f/tPSA, comorbidity, educational level, and medication use. Outcomes (dependent variables) were both prostate cancer in general and high-grade prostate cancer defined as a Gleason score of ≥ 7. When analyzing high-grade prostate cancer, low-grade cancers were classified as controls together with benign findings. When analyzing subgroups of medications, other subgroups were excluded; for example, when analyzing the effect of *hydrophilic* statins, men using other statins were excluded from the analysis.

5 RESULTS

5.1 PAPER I

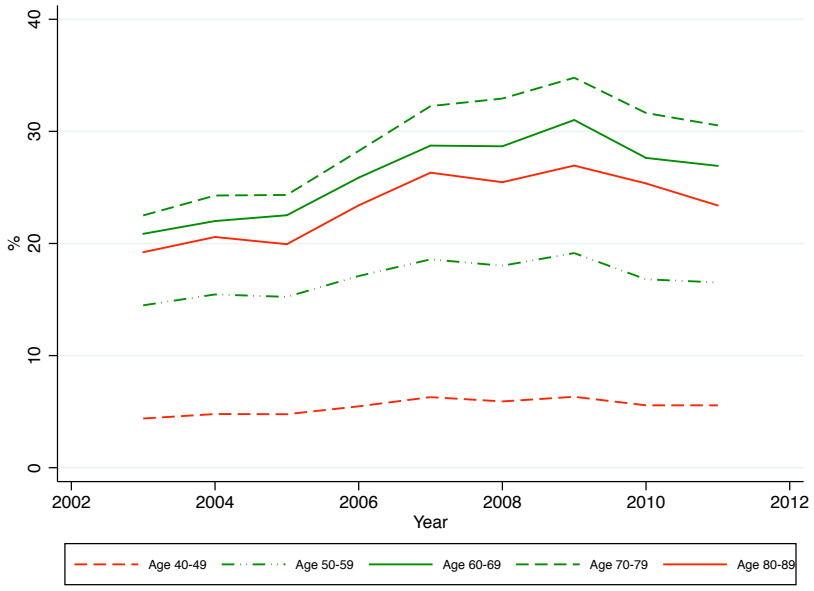
In the male population of 1,034,129 men in Stockholm 2011, we identified 229,872 who had had a PSA test between 2003 and 2011. Median age for men having a PSA test was 64 years. We restricted the analysis to men aged 40–89 years and the prevalence of having a PSA test is illustrated in table 10.

Table 10: Population of Stockholm County by age group, and 12-month and 9-year prevalence proportions of PSA testing in Stockholm County, 2011

Prevalence of PSA testing in Stockholm County, 2011			
Age group years	Population n	PSA in the last 12 months	PSA in the last 9 years
50–59	121,260	16.5%	45.5%
60–69	103,344	26.9%	67.7%
70–79	50,266	30.5%	77.1%
80–89	22,546	23.4%	72.5%
50–89	**297,416**	**23.0%**	**60.6%**

We addressed historical PSA testing by calculation of 1-, 2-, and 5-year prevalence over the period 2006–2011. As illustrated for 1-year prevalence in Figure 6, it became increasingly common to come for PSA testing until 2009, after which testing decreased somewhat.

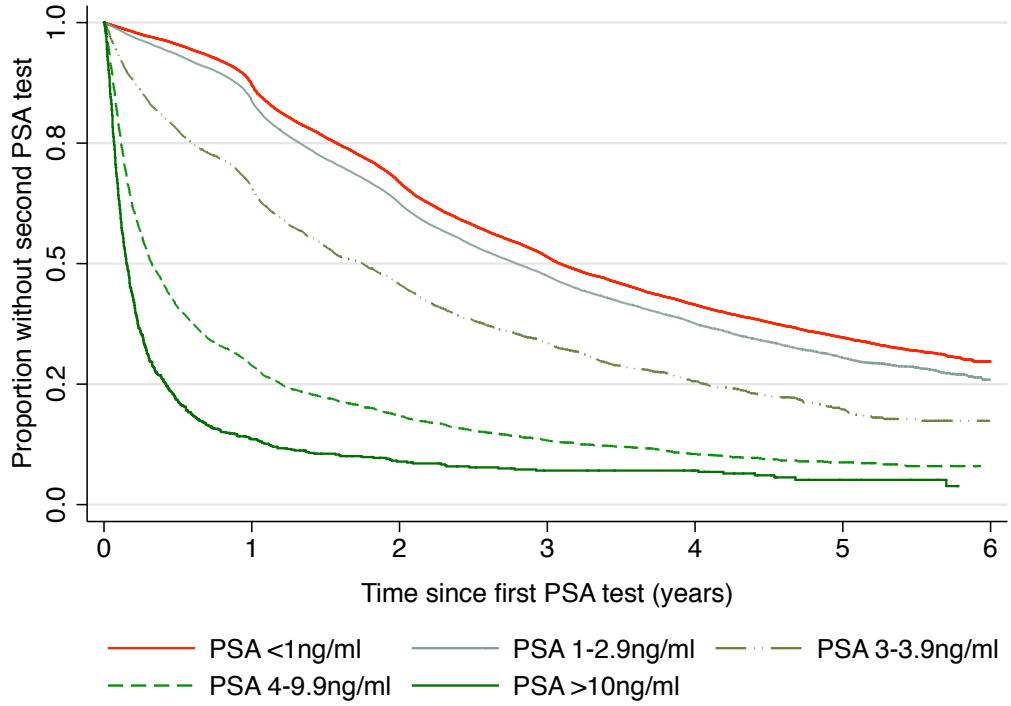
Figure 5: One-year prevalence proportion of PSA testing for men in Stockholm, 2011. Red: Age groups uncommon to treat with curative intent (Age 80-89) or with low cancer incidence (Age 40-49).



Frequent returns for second and third PSA tests can be seen as proxies for screening behavior. We found that almost half of the men over 50 years of age returned within

two years for a second test irrespective of the original PSA value. Strikingly, a third of these men will return within two years even if their first PSA value is below 1 ng/ml, which corresponds to a very low risk of long-term metastasis or death from prostate cancer [33,34]. Furthermore, of the men aged 80–89 who would not gain from curative treatment of a prostate cancer, 52% came back for PSA testing within 2 years. We can illustrate this behavior by plotting the proportion of men without a second PSA test in men without previous prostate biopsy or prostate cancer diagnosis (Figure 6).

Figure 6: Proportion of men not having a second PSA test. Survival function for time between first and second PSA test. Men with previous biopsy or PCa excluded. Red: Men with PSA < 1ng/ml.



5.2 PAPER II

In 172 men with PSA levels of 1–3 ng/ml who had not previously had a prostate biopsy or prostate cancer diagnosis, we found prostate cancer in 27% (n = 47). Ten (6%) of the men had a tumor with a Gleason score of at least 7. After two years of follow-up, 14 (8%) had had curative treatment (reviewed by me in August 2014). Median length of cancer lesion in men with prostate cancer was 2 mm (Table 11).

Although the numbers were too small to evaluate statistically, they indicated a twofold risk of high-grade cancer in the high-risk group as compared to the population average (12% vs. 6%).

Table 11: Results of first prostate biopsy in 172 men with PSA 1–3 ng/ml selected according to genetic risk score category

	Genetic risk score category			
	Low risk	**Intermediate risk**	**High risk**	**Total**
No. of participants	50	79	43	172
Prostate cancer, % (n)	18 (9)	28 (22)	37 (16)	27 (47)
Gleason 6, % (n)	16 (8)	22 (17)	26 (11)	21 (36)
Gleason ≥ 7, % (n)	2 (1)	5 (4)	12 (5)	6 (10)

Table 12 shows the logistic regression models where the genetic score was associated with the risk of prostate cancer (OR = 1.60). Small prostate volume was associated with an increased risk of prostate cancer, but the f/tPSA ratio was not. The cohort was selected according to tPSA and age, and as might be expected these variables were not significantly associated with outcome.

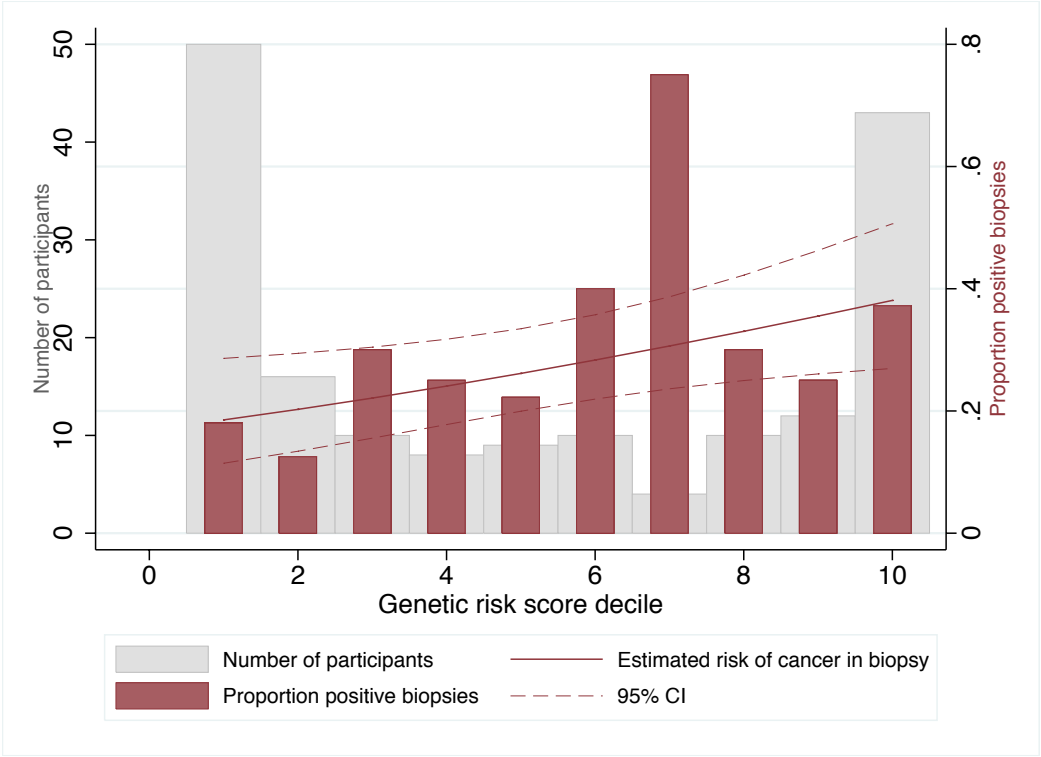
Table 12: Logistic regression regarding risk of prostate cancer in 172 men aged 50–69 years with PSA levels of 1–3 ng/ml. Men with previous PCa were excluded. Family history was defined as any first -degree relative with PCa

	Univariate			Multivariate		
Risk factor	OR	CI (95%)	p-value	OR	CI (95%)	p-value
Genetic score	1.67	1.11–2.50	0.014*	1.60	1.05–2.45	0.029*
Total PSA, ng/ml	1.04	0.76–1.40	0.83	1.06	0.72–1.54	0.78
Free PSA / total PSA	0.97	0.94–1.01	0.16	0.98	0.95–1.02	0.42
Age, years	1.05	0.97–1.14	0.21	1.08	0.99–1.18	0.10
Prostate volume, cm^3	0.96	0.92–0.99	0.013*	0.95	0.91–0.99	0.010*
Family history (yes/no)	0.60	0.25–1.41	0.24	0.73	0.29–1.81	0.49

OR, odds ratio; CI, confidence interval.

Figure 7 illustrates the observed proportion (red bars) and calculated proportion (red line) of men with cancer findings according to their genetic score. The gray bars show the number of participants in each risk decile.

Figure 7: Observed risk (red bars) and estimated risk (red line) of a positive biopsy in 172 men aged 50–69 years with PSA 1–3 ng/ml, according to decile of genetic risk . Genetic risk was assessed by a genetic score based on SNP. Gray bars: sampling distribution of study subjects.



5.3 PAPER III

Altogether, 531 participants (271 cases and 260 controls) with PSA levels of 3–15 ng/ml were included in the main analysis. Both PHI and the four-kallikrein panel showed higher AUC than the base model, both when predicting all prostate cancer and when predicting high-grade prostate cancer. However, as seen in Table 13, there was no significant difference in AUC between PHI and the four-kallikrein panel.

Table 13: Performance of models predicting prostate cancer when applied to a cohort of 531 previously unbiopsied men with PSA 3–15 ng/ml

	All cancer			High-grade cancer (Gleason 7+)		
Model	**AUC**	**95% CI**	**p-value**	**AUC**	**95% CI**	**p-value**
Base model	54.5	(49.6–9.4)	Ref.	59.6	(54.1–65.8)	Ref.
Four-kallikrein panel	69.0	(64.5–73.4)	< 0.01	71.8	(66.8–76.7)	< 0.01
PHI	70.4	(66.1–74.8)	< 0.01	71.1	(66.0–76.2)	< 0.01
AUC comparison:						
PHI vs four-kallikrein panel			0.52			0.77

Compared to a strategy where the entire cohort is biopsied (as in clinical practice today), both PHI and the 4K model avoided biopsies. Using 4K model = 10% and PHI = 39 as the cutoff for biopsy, both models saved about a third of the biopsies at the cost of missing every tenth high-grade cancer, as illustrated in Table 14.

Table 14: Head-to-head evaluation of the four-kallikrein panel (4K) and the PHI model in terms of saved biopsies and missed cancers compared to a strategy where the entire cohort is biopsied

	Biopsies		**All cancers**		**High-grade cancers**	
Cutoff	Performed	Saved	Missed	Risk of cancer in unperformed biop.	Missed	Risk of high-grade cancer in unperformed biopsies
	n	%	%	%	%	%
Biopsy all	1,000	0.0%	0.0%	NA	0.0%	NA
4K						
10%	704	29.6%	16.3%	28.0%	10.5%	9.5%
15%	550	45.0%	30.0%	34.0%	21.8%	12.9%
20%	437	56.3%	44.7%	40.5%	36.5%	17.2%
PHI						
26	915	8.5%	2.5%	15.3%	4.5%	14.1%
39	704	29.6%	15.1%	26.0%	9.8%	8.8%
47	550	45.0%	30.6%	34.7%	24.4%	14.4%

5.4 PAPER IV

A total of 185,657 men without previous prostate cancer or prostate biopsy had their first PSA taken in Stockholm County during the period 2007–2012 and were included in the study. The prevalence of low-dose aspirin, statin, and anti-diabetic medication was 12%, 12%, and 4%, respectively. Of these men, 18,574 were identified to have a first prostate biopsy after the PSA: 54% had benign biopsy findings, 17% and 23% had prostate cancer findings on biopsy with Gleason scores of ≤ 6 or ≥ 7, respectively. A comparison of PSA levels between users and non-users of the medications is given in Table 15.

Table 15: Relationship between use of medications and PSA concentration in Swedish men undergoing their first PSA test and having no prior prostate cancer or prostate biopsy

Medication	% tPSA difference (users vs. non-users)	95% CI	p-value
Aspirin	−3.4%	−5.2 to −1.7	< 0.001
Statins	−4.6%	−6.2 to −2.9	< 0.001
Metformin	−15%	−18 to −12	< 0.001
Insulin	−16%	−19 to −13	< 0.001

In multivariable regression analysis, increasing age, increasing PSA level, and decreasing f/tPSA were all associated with finding prostate cancer in general and high-grade prostate cancer. Statin use was associated with an increased risk of any prostate cancer and high-grade prostate cancer (OR = 1.16 and 1.25; 95% CI: 1.04–1.29 and 1.10–1.42). This effect persisted in subgroup analysis of both hydrophobic and hydrophilic statins. No significant association between prostate cancer and aspirin (OR = 1.1 and 1.03) or metformin (OR = 1.0 and 1.2) was seen—regarding any prostate cancer or regarding high-grade prostate cancer.

6 METHODOLOGICAL CONSIDERATIONS

6.1 PAPER I

Paper I was a descriptive, population-based study on the PSA testing behavior in Stockholm County. STHLM0 includes data on all PSA-tested men from all the three known laboratories in Stockholm County (Karolinska university Laboratories, Aleris, Unilabs), making conclusions at the population level possible. However, there is always the possibility that another laboratory in the area provides PSA tests. In search of PSA laboratories, we have repeatedly gathered information from both clinicians and laboratory personnel, but have found no evidence of other PSA laboratories in Stockholm County.

Secondly, the study did not include data on men moving to Stockholm County after undergoing PSA testing outside the region, if they had no subsequent PSA test. Furthermore, there is a small chance that some Stockholm men get PSA-tested outside the region, e.g if living in the periphery of Stockholm. This proportion is difficult to estimate, but exclusion of the PSA tests on these men gives conservative estimates of the testing levels. Assuming that immigrant men had testing patterns similar to those of Stockholm men, we performed sensitivity calculations on the effect of migration and concluded that the underestimation of the 9-year testing prevalence was < 1.5%.

Thirdly, Paper I does not provide any knowledge on why men or their physician decide to test while lacking information on the reason for testing. There are a number of situations where it is reasonable to test for PSA in older men or men with low PSA levels previously, e.g if there are symptoms suggestive of advanced disease. This study provides no means of differentiating these men from asymptomatic men coming for opportunistic screening.

Finally, the study provides a description of the use of PSA tests in only one urban Swedish region with high opportunistic testing, and says less about national or international testing behavior. In the setting described, there is a certain amount of unnecessary testing. The test prevalence described is in line with previous studies on national PSA testing using cancer incidence as a proxy for PSA testing [135], increasing the validity of both studies.

6.2 PAPER II

Paper II was a prospective study investigating whether a genetic score gives predictive information about prostate biopsy results in men with low PSA values who are not biopsied in current clinical practice. It provides information on a group of men for whom evidence for level of performance of most biomarkers that have been suggested is lacking. Biopsying urologists and the single pathologist were blinded regarding the genetic score, reducing the risk of detection bias. However, the study had certain limitations. First and foremost, men were selected from the STHLM2 cohort, in which they were included when giving blood for a PSA test. Thus, the participants first had a PSA test for an unknown reason, then accepted being included in the STHLM2 cohort,

and finally agreed to undergo a prostate biopsy knowing that this would not have been done in clinical practice. It is likely that men with relatives with prostate cancer would be over-represented in such a selection process. Thus, the participants could hardly be expected to represent the general male population, possibly introducing selection bias. As family history was easily accessible, we incorporated this in the regression analysis, finding that the genetic score showed independent value besides the family history information. Apart from family history creating selection bias, there is no obvious reason why men in the study would differ from men in the Swedish population regarding the association between harboring SNPs (the genetic score) and prostate cancer. However, the association described must be interpreted with caution when dealing with international populations.

Secondly, the study lacked power to analyze the association between genetic score and high-grade cancer, which is perhaps more interesting than the association with prostate cancer in general. The finding of 1, 4, and 5 high-grade cancers in the low, intermediate, and high genetic risk groups is interesting, but the numbers are too small for statistical analysis. Due to ethical considerations and limited resources, the study was powered to show differences in detection of overall cancer. Thus, the clinical implications of the findings remain unclear. The clinical benefit when comparing a base model with a model where the genetic score was added was roughly estimated using net reclassification improvement (NRI), which turned out to be positive but statistically insignificant. Larger studies are needed to address both the association between the genetic score and high-grade cancer, and the clinical usefulness of this score.

6.3 PAPER III

In Paper III, the performance of PHI and the four-kallikrein panel was evaluated in the STHLM2 cohort. The paper provides the first direct comparison of these two kallikrein-based models, showing strikingly similar performance. Together with most biomarker studies, our comparison lacked data on men with low PSA values. Furthermore, since all men were biopsied according to current clinical practice, use of the models will provide a means of saving some men from undergoing biopsy, but only at the cost of missing some cancers that would otherwise have been found in current practice (assuming that none of the models are “perfect”). However, there is no obvious reason why the similarities in performance would change drastically in men with low PSA, making it possible that the four-kallikrein panel and PHI would perform similarly in general. The actual performance of these models in men with low PSA is, however, still not known.

Neither DRE, prostate volume, nor family history were available. As these are often considered in clinical biopsy decisions, this may have introduced selection bias into the study, adding to the possible selection bias due to inclusion into the STHLM2 cohort mentioned above. However, the participants were all men in Stockholm County being biopsied in clinical practice after inclusion in STHLM2, making it a relevant cohort of men to explore.

Finally, f/tPSA is in clinical use in Sweden, making use of biomarker panels incorporating this performing relatively less well in a Swedish cohort. That is, some of the biopsies were probably decided upon using f/t PSA information. It could then be expected that the models explored would perform less well than in a setting where only tPSA is used.

6.4 PAPER IV

Paper IV was a retrospective, population-based study exploring the association between use of three pharmaceutical drugs on the one hand and PSA levels and the risk of finding prostate cancer from biopsy on the other. There are apparent risks of bias in all observational studies, and this study had several limitations. Firstly, we lacked information on the indication for PSA testing in men in Stockholm, giving a risk of selection bias. However, two-thirds of men aged > 50 years undergo PSA testing in this region (Paper I). This suggests that the difference in testing between men on aspirin, for example, and men without medication could be limited. To investigate the possible effect of this on PSA level estimations, we adjusted the linear regression for both age and comorbidity, including only the first known PSA test in each man.

Secondly, it is possible that men on medication seek biopsy more or less often than others. With this, there is a risk of introducing detection bias that masks potential protective effects of the medication. If so, it could follow that we would find more insignificant cancer in men on medication if they are biopsied more frequently. To address this, we only included men coming for their first biopsy, and our logistic regression model included both age, comorbidity, education, PSA levels, and use of medications.

Thirdly, there was a risk of exposure misclassification as we lacked data on medications bought over the counter. Since non-prescription use will probably continue also in a setting with organized chemoprevention, we believe that the situation reflected is relevant.

Finally, as in other observational studies, residual confounding may have been introduced by unmeasured risk factors for prostate cancer (e.g. physical activity, diet, and body mass index). In summary, our study shares apparent risks of bias and residual confounding with previous observational studies on the subject, which possibly explains the wide-ranging estimates of the associations between these drugs and prostate cancer.

7 DISCUSSION

7.1 PSA TESTING BEHAVIOR

7.1.1 PSA testing in men aged 50–69 years

In Paper I, we found that PSA testing and retesting in Stockholm County, where PSA screening is not recommended, was common in all men aged over 50 years and that it was uncommon in younger men. How one should appraise this depends very much on the age and PSA level of the men being tested. Men aged below 70 have the clearest benefit from treatment with radical prostatectomy for prostate cancer, and the ages 50–69 years are often suggested to be relevant for prostate cancer screening [136]. We showed that 46–67% of men in this age group had had a PSA test during the previous 9 years and that 25–40% had had a PSA test during the previous two years. When addressing retesting, we note that half of all men in this age group return for another PSA test within three years, even if they have had an original PSA value below the traditional threshold for prostate biopsy (3 ng/ml). The intensity of PSA testing in Stockholm County is almost comparable to participation rates in the screening arm of the ERSPC screening trial (82%), but without the positive attributes of an organized screening program [90]. With this in mind, it is reasonable to state that there is frequent, unorganized testing in Stockholm County in men who are relevant to organized prostate cancer testing programs.

7.1.2 PSA testing in elderly men

One of the suggested golden rules for prostate cancer screening is: “Don’t screen men who won’t benefit” [98]. No guidelines support early detection of prostate cancer with PSA in men over 75 years old, and indeed there is no mortality-related benefit of treating men much over 70 years curatively for prostate cancer [136,137]. It has been estimated that almost half of all prostate cancers detected in screening programs represent over-detection [138]. Screening for localized prostate cancer in men over 70 years of age could result in even higher proportions, giving a high risk of unnecessary treatment.

Limited use of PSA testing does serve a role in elderly men with symptoms or palpatory findings on rectal examination suggestive of advanced prostate cancer. These men are, however, few—especially when compared with the number of men with benign voiding symptoms. Approximately 20% of men aged > 75 years have lower urinary tract symptoms (LUTS) or a diagnosis of benign prostatic hyperplasia (BPH) [139]. Although EAU states that PSA measurement should be performed only if a diagnosis of prostate cancer will change the management of BPH [140], such symptoms possibly drive PSA testing.

We found that in all age groups, PSA testing is most common in men aged 70–79 and 80–89 years where two out of three men have had their PSA taken in the last five years (in both cases). Even more illustrative is the fact that every fourth man aged 80–89

years has had his PSA taken in the last 12 months. This means that 31% (27,551 out of 89,209) of men aged 40–89 years who were tested in Stockholm County in 2011 were over 70 years of age, illustrating that unnecessary and probably harmful testing is common.

Moreover, as PSA testing is not organized centrally, PSA testing rates can vary between doctors. In a US setting, a 10-fold difference in PSA testing rates in men aged more than 75 years has been shown between the 10% of primary care physicians testing most and least often [141]. The high and variable level of PSA testing in elderly men calls for organized testing or improvement in guidelines.

7.1.3 Retesting in men with low baseline PSA level

With regard to studies on the low long-term risk of prostate cancer metastasis and death in men with low baseline PSA in mid-life, it is relevant to focus also on retesting habits in men with low PSA levels [33,34,97]. EAU guidelines have used this knowledge, recommending a retest interval of 8 years if baseline PSA is < 1 ng/ml [4]. In this light, it is remarkable that every third man in Stockholm County with PSA< 1 ng/ml has a second PSA test within 2 years. On the other hand, approximately 30% and 10% of men with PSA< 1 ng/ml and 4–10 ng/ml respectively have *not* returned for subsequent PSA testing within 6 years, indicating that there probably still is a proportion of men without reasonable follow-up. This frequent retesting behavior is pronounced in all age groups over 60 years, with lower frequency of retesting in younger men.

Frequent testing in elderly men and men with low baseline PSA drive over-diagnosis of cancers that would not affect the man if untreated, and over-treatment where men are subjected to treatment that does not prolong their life but has negative effects on quality of life [96]. Furthermore, there is an unknown health-economic cost related to this unnecessary testing. Our findings contrast with recent knowledge and existing guidelines, and may have a profound impact on the organization of future prostate cancer testing.

Table 16: Findings in Paper I that contrast with current guidelines. Study population: men in Stockholm County without a diagnosis of prostate cancer.

Age group, years	**PSA level,** ng/ml	**Test prevalence,** %	**Cumulative incidence of second PSA test within 26 months,** %
70–79	all	67.9 (last 5 years)	57.5
80–89	all	72.5 (last 9 years)	52.8
40–89	< 1	N.A.	33.7

7.2 UNDETECTED PROSTATE CANCER IN MEN WITH LOW PSA

A major drawback of most biomarker studies for detection of prostate cancer is that all men undergoing biopsy in most studies are chosen due to their elevated PSA, although every fourth man with PSA 1–4 ng/ml has prostate cancer and 2–7% of them have prostate cancer of Gleason grade ≥ 7[32].

Using data from STHLM0 and Paper I (see section 4.1), we can calculate the number of men aged 60–69 in Stockholm County by PSA strata (proportion of PSA-tested men within stratum times the size of the male population). Assuming that the proportions of prostate cancer are consistent between the Swedish and the predominantly white US population used in the PCPT trial, we can then estimate the number of Stockholm men aged 60–69 years with undetected prostate cancer by PSA stratum, as shown in Table 17. The validity of this assumption is supported by findings in Paper II (6% Gleason ≥ 7 tumors in men with PSA 1–3 ng/ml).

Table 17: Estimated number of men aged 60–69 years in Stockholm County that harbor prostate cancer. High-grade prostate cancer was defined as Gleason score ≥ 7

STHLM0 data		**Data from Thompson et al. [32]**			
PSA, ng/ml	**Est. men in population,** n	**Men with PCa,** %	**Men with high-grade PCa,** %	**Est. men with PCa,** n	**Est. men with high-grade PCa,** n
1.1–2.0	28,461	17%	2.0%	4,838	571
2.1–3.0	13,238	24%	4.7%	3,164	626
3.1–4.0	7,472	27%	6.7%	2,010	502
All psa	103344				

PCa, prostate cancer; Est., Estimated.

From Table 17, it follows that approximately 1% ([571+626]/103,344) of all men in Stockholm aged 60–69 years would be diagnosed with high-grade prostate cancer if biopsied, though having a PSA below a commonly used threshold for biopsy (3 ng/ml). These men are under-diagnosed today and are at risk of developing advanced disease.

Furthermore, while there are more men with PSA levels of 2–3ng/ml than men with 3–4 ng/ml, in absolute terms there are approximately 20% (626 vs. 502) more men in the lower range who have high-grade tumors than in the higher range—even though the risk of cancer is higher with increasing PSA.

7.3 PERFORMANCE OF BIOMARKER MODELS

7.3.1 Detection of prostate cancer in men with low PSA

In order to reduce over-diagnosis, over-treatment, and biopsy-related morbidity, it is of utmost importance to reduce the number of biopsies performed. Only this would effectively reduce the number of insignificant tumors detected and the number of post-biopsy infections due to antibiotic-resistant bacteria.

Virtually all studies of suggested biomarkers in prostate cancer use samples from cohorts of men who have undergone prostate biopsy based on their age and tPSA level. These studies can therefore only lead to conclusions regarding settings in which men are first chosen on the basis of PSA level and age, and only thereafter is the new tool applied. In this context, a new biomarker can only save biopsies at a certain cost (missed cancers) while all men would be biopsied in current practice (they were all chosen based on PSA level) and no suggested biomarker has yet been perfect (perfectly identifying only men with cancer).

One solution to detection of as many high-grade cancers as today or more, and still be able to decrease the number of biopsies is to find men with a high risk of prostate cancer among men who are not biopsied today. This can be done either through (1) better organized testing in order to find men who are not tested at all in current practice or (2) also testing for prostate cancer in men with low PSA in order to detect the high-grade cancers described above (section 7.2).

7.3.2 Genetic score

With genotyping being increasingly available and cheap, this opens up a way of estimating an individual's risk of prostate cancer originating from genetic factors. Single-nucleotide polymorphisms (SNPs) now have the advantages of being cheap, stable (need only to be measured once), and easy to analyze. The individual increase in risk if carrying an individual nucleotide polymorphism is low, but the 100 nucleotide polymorphisms known to be associated with prostate cancer explain about a third of all familial risk [70,142,143]. Previously, the STHLM1 study used a genetic score based on the then validated 35 SNPs, indicating that roughly every fourth biopsy procedure could be avoided at the cost of missing 8% of cancers of Gleason score ≥ 7 [72]—if the genetic score was applied to men biopsied according to current clinical practice. A calculation was also performed estimating that the proportion of saved biopsies could increase to every third biopsy if all SNPs associated with prostate cancer were known.

We performed the study in Paper II in order to determine whether the genetic score was also informative regarding the risk of cancer in men with low PSA, thereby giving the possibility of identifying men with prostate cancer not detected in today's routines. In 172 previously unbiopsied men aged 50–69 years with a PSA level of 1–3ng/ml, we found that 27% and 6% harbored prostate cancer and high-grade prostate cancer (Gleason score ≥ 7) respectively.

We found an increased risk of prostate cancer with increasing genetic score (OR =

1.60; 95% CI: 1.05–2.45) with an absolute increase in risk of 10 percentage points (37% vs. 27%) for men in the highest genetic risk decile, as compared to the population average. The Gleason ≥ 7 tumors were distributed as 2%, 5%, and 12% in the low-, intermediate-, and high-risk groups, respectively. This might indicate a twofold increase in risk (12% vs. 6%) in men in the high-risk group compared to the population average, but the numbers are too small for proper statistical evaluation.

The adjusted association between the genetic score and risk of prostate cancer was similar in this study to that in a previous study on men with higher PSA (OR = 1.60 vs. 1.52), possibly indicating that the genetic score performs equally well in all PSA strata. Though providing independent information, the modest increase in AUC when comparing previously published genetic score models with clinical models based on currently available variables (age, family history, tPSA, f/tPSA, prostate volume) might indicate that the optimal biomarker panel for predicting risk of prostate cancer should also incorporate additional variables.

7.3.3 PHI and the four-kallikrein panel/4Kscore

Both the Prostate Health Index (PHI) and the four-kallikrein panel (4Kscore) represent multiplex protein biomarker models predicting the risk of prostate cancer in terms of an index (PHI) or percentage risk of outcome (4Kscore). PHI is a commercially available, FDA-approved test (Beckman Coulter Inc.) and the 4K panel is commercially available (4Kscore; OPKO Health Inc.), but is not FDA-approved. Instead, it is offered as a laboratory-developed test (LDT).

Both tests were developed to aid biopsy decisions and they have been validated separately in men undergoing biopsy in current practice, thus having at least moderately increased PSA. In men coming for first biopsy, both models have repeatedly shown an independent additional value when added to current clinically available information (age, prostate volume/DRE, tPSA, f/tPSA). The individually used risk cutoff could be chosen according to patient/doctor preference, and the clinical usefulness of avoiding biopsies is dependent on the cutoff chosen for biopsy. PHI = 27 is a suggested cutoff for PHI and a 20% risk of high-grade cancer is often used to illustrated 4Kscore performance. Using the 4Kscore, a third to half of all biopsies can be avoided at the cost of missing every tenth to twentieth high-grade cancer. For PHI, it has been reported that 15% of biopsies can be avoided at to the cost of missing only a few percent of high-grade cancers. Thus, the use of PSA derivatives and additional kallikrein markers has the potential to improve the current performance characteristics of the PSA test alone [144]. However, there has been a lack of comparisons between these two tests with apparently similar characteristics.

We used a population-based cohort of 531 men with PSA 3–15 ng/ml and prostate cancer or benign findings on a first prostate biopsy to provide an external validation and head-to-head comparison between PHI and the four-kallikrein panel (4Kscore). Both tests independently improved discrimination for both prostate cancer and high-grade disease compared to a base model, and there was no significant difference in discrimination between the tests. Since a prostate cancer test must have high sensitivity,

we also compared partial AUC when sensitivity exceeded 75%. In this analysis also, there was no significant difference in discrimination between the two models.

The finding that PHI and the four-kallikrein panel performed comparably well in predicting all prostate cancer and high-grade prostate cancer is further reflected in the analysis of potentially saved biopsies. In order to avoid 30% of biopsies, a cutoff of 10% for the four-kallikrein panel and PHI = 39 was determined. When using these cutoffs, 10.5% and 9.8% of high-grade cancers, respectively, were missed.

In this dataset, previously suggested phi cutoffs (25–30) corresponded to a low risk of prostate cancer and PHI in the range 30–40 had better properties. This was also reflected in calibration curves where PHI was poorly calibrated to previously reported associations between prostate cancer risk and PHI level. The four-kallikrein panel was well calibrated, both for predicting high-grade prostate cancer and all prostate cancer.

In summary, PHI and the four-kallikrein panel performed similarly, with small discrepancies in calibration characteristics. As both biomarker models contain measurements of biologically closely related kallikreins, this finding may not be surprising. Sensitivity analyses including men with higher PSA and previous biopsy did not alter the results materially. From a clinical point of view, this is reassuring, since previous biopsy status may not always be known—and since the models are also of potential value for deciding on prostate biopsy in men with PSA levels outside the 3–15 ng/ml range.

7.4 CHEMOPREVENTION OF PROSTATE CANCER

Because no other study design can provide the safeguards against bias associated with randomization, randomized controlled trials (RCTs) are essential to provide a basis for recommendations on prevention of cancer. The inability of observational studies to control for unknown prognostic factors leads to a risk of biased conclusions. Therefore, observational studies are classified as providing weaker evidence than RCTs [145]. However, RCTs consume vast amounts of resources and other study designs can be used to determine whether to initiate a trial.

An example of discripancies between results of observational studies and randomized trials is the controversy surrounding postmenopausal hormonal replacement therapy (HRT). Numerous observational studies had appeared that indicated that HRT was “good for you”, which is why a very large randomized trial (Women’s Health Initiative; WHI) was initiated. WHI recruited 16,608 post-menopausal women that were randomized to combined estrogen/progesterone-treatment or placebo. Surprisingly, this indicated that a combined regimen of estrogen and progesterone increases the risk not only of breast cancer (HR 1,26) but also of cardiovascular disease (HR 1.22), and the trial was stopped after five years due to the association between breast-cancer and HRT[146,147].

Pharmaceutical drugs already in common use are interesting and feasible to explore for chemoprevention, for a number of reasons. Firstly, the security profile is already known and rare side effects are also hopefully reported. Secondly, generic medications or at least cost-effective alternatives might be available. Finally, assuming that the condition the drug is in current use for is not associated with the development of cancer, the association of medication with cancer risk can be explored through observational studies, often using existing data from registries or previous studies on other subjects.

Aspirin and statins are widely used for prevention of cardiovascular disease, have a relatively benign security profile, and are cost-effective. A number of observational studies have found associations between aspirin, statin, and metformin use and the risk of prostate cancer (see section 1.5). A modest inverse association between the risk of prostate cancer and aspirin or statins has been reported, but the evidence is conflicting. Furthermore, there is no clear dose-response effect. Metformin is a potent anti-diabetic medication with somewhat more side effects, but even so, RCTs have been suggested based on findings in some retrospective studies [148].

As we are well aware that point estimates of observational studies have, historically, differed substantially from those of subsequent trials, RCT initiatives on aspirin, statins, or metformin are mandatory if considering prevention of prostate cancer with those drugs. In order to decide on whether to support such initiatives, we used a Swedish population-based cohort to provide additional retrospective evidence on the association between aspirin, statins, or metformin and the risk of prostate cancer. When including socioeconomic data, comorbidity data, PSA levels, and clinical data, we found no inverse association between these medications and the risk of finding cancer or high-grade cancer on biopsy; on the contrary, we found an increased risk of cancer in men on statin therapy.

In line with previous studies, we found slightly lower PSA levels in men on aspirin, statin, or anti-diabetic medication, which would possibly affect the results of observational studies without access to PSA data.

The population-based nature of the STHLM0 cohort, the independent ascertainment of exposure data, and access to original PSA and biopsy data gave strength to the study. In summary, we found no support for initiation of a trial on the relationship between these drugs and the risk of prostate cancer. As further discussed in the methodological considerations (section 7.5), we acknowledge the risk of several kinds of bias and confounding associated with observational studies.

7.5 FUTURE PERSPECTIVES

7.5.1 Organized prostate cancer testing

A large proportion of Swedish men undergo testing for prostate cancer, as illustrated in Paper I. The same has previously been shown in other nationalities to varying degrees [149-151]. While this unorganized screening behavior is present, I agree with the Editorial on Paper I, stating that “screening for prostate cancer is likely here to stay”

[152]. Also, the majority of authors in the recent Lancet Oncology review on early detection of prostate cancer stated that "PSA screening does reduce death from prostate cancer" [153]. This is supported by the findings in the ERSPC trial, showing a substantial reduction in prostate cancer mortality in men in the PSA screening group. In this trial, the number needed to invite (NNI) to prevent one death from prostate cancer was roughly 800, being substantially lower than commonly reported NNIs for breast cancer screening (NNI 1339-2000) [90]. From this, it is not reasonable to believe in implementing a complete stop in prostate cancer testing in asymptomatic men.

However, as discussed in section 2.4, this is a matter of debate—where both USPTF and the Canadian CTFPHC recommend against screening with PSA because of the high degree of over-diagnosis and common side effects of diagnosis and treatment (biopsy complications, postoperative incontinence/impotence, post-radiation incontinence/bowel dysfunction/impotence) [100,101].

We have shown how the unorganized testing situation in Sweden drives testing in men who do not benefit from it due to age or PSA level. Organized testing programs are a way of reducing test-related harm—such as over-diagnosis and over-treatment—in these men. An often overlooked feature of the screening debate has been that screening trials only study testing *within* the study population (e.g. men aged 50–69 years). If implementing an organized program and thereby also controlling testing outside the age range relevant to screening, test-related harm *outside* this age range could be reduced (e.g. in elderly men).

The goal of a testing program is, however, not necessarily a 100% participation rate. Rather, the objective of an organized prostate cancer testing program should be to (1) invite men who will potentially benefit from testing, aiming to include only well-informed and willing men, and (2) avoid testing in men who would not benefit from it.

7.5.2 Validation of suggested biomarkers

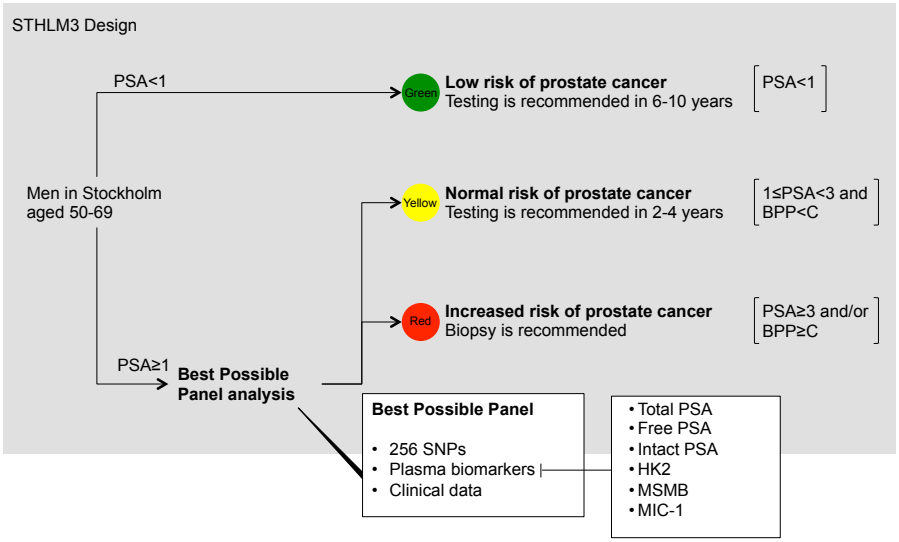
In order to detect as many high-grade cancers with a risk-assessment tool as when using a cutoff for biopsy based only on age and PSA, men with lower PSA than this cutoff must be biopsied. For this, new biomarkers must be validated in study populations including men with low PSA. With this, it follows that new screening trials, where men are not selected merely based on levels of PSA in the experimental arm, will be needed.

The STHLM3 trial (www.sthlm3.se) addresses the question of whether a biomarker panel based on age, family history, biopsy history, protein levels (kallikreins, beta-microseminoprotein, growth differentiation factor 15 etc.), and genetic information (single-nucleotide polymorphisms) can save prostate biopsies while maintaining the sensitivity for high-grade disease. It is currently being performed in Stockholm and uses a paired design where men aged 50–69 years with a high risk of prostate cancer (as judged by a PSA cutoff of 3 ng/ml or the biomarker panel) are invited for prostate biopsy (See Figure 9, Red). Included men with a normal or low risk

of prostate cancer are recommended retesting in line with current knowledge. The STHLM3 trial has included more than 55,000 men (November 2014), and will publish preliminary results in March 2015.

The STHLM3 trial introduces prostate biopsy in men who do not come for biopsy in current practice. Thus it explores the idea of finding more cancers in men with low PSA in order to maintain sensitivity for high-grade tumors while avoiding biopsies in men with moderately increased PSA. Adequate validation of future additional biomarkers should ideally relate to this concept.

Figure 8: The design of the STHLM3 trial.



7.5.3 A new diagnostic pipeline for prostate cancer

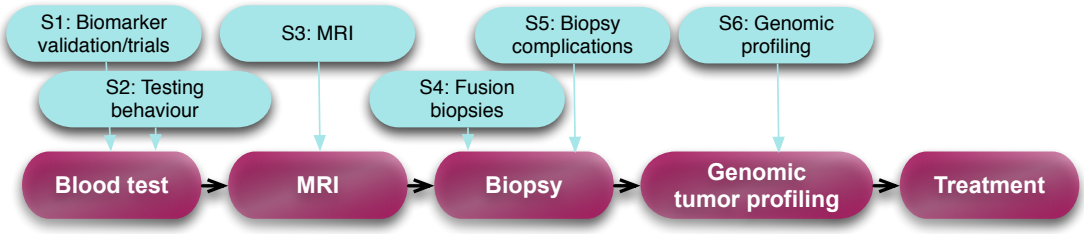
Today, the situation in prostate cancer diagnostics is marked by frequent, unorganized testing with an inadequately performing biomarker (PSA). For men with increased PSA, this is followed by systematic (and not targeted) biopsy— finding (at least to some degree) insignificant tumors and causing increased infectious complications. Treatment decisions are then often based on biopsy findings in terms of Gleason score and extent of spread together with findings on MRI.

In order to enhance the performance of the diagnostic pipeline for prostate cancer, a number of measures can be taken. One possible direction forward could be accompanied by studies at several levels, as outlined in Figure 9. Firstly, an improved blood test used in men who would benefit from testing must be established. This would need both validation studies of existing biomarkers—as discussed above, especially in men not undergoing prostate biopsy in today's practice—and development of new biomarkers to be validated later (S1). Furthermore, testing behavior in men needs to be clarified further, e.g. by addressing current PSA testing habits in men according to socioeconomic factors and comorbidity (S2).

Secondly, for men being treated for prostate cancer, it is already increasingly common to undergo MRI. Current evidence indicates that MRI-targeted biopsies - either directing the biopsy needle through fusion with ultrasound or direct in the MRI tube - can reduce the number of insignificant tumors and the number of performed biopsies while increasing the numbers of detected significant tumors [88]. With this follows reduced morbidity due to the infectious complications of prostate biopsies. However, the concept of performing MRI *before* biopsy to target the tumor needs to be validated, both in men coming for initial biopsy and in those coming for re-biopsy (S4). The continuously evolving performance of modern MRI done before targeted biopsies in guiding definitive treatment also needs further validation (S3). Meanwhile, it is of utmost importance that current biopsy practice should be updated in line with the increasing numbers of infectious complications with antibiotic-resistant bacteria. Continuous improvement of biopsy technique is warranted and might include both cross-sectional studies on current practice and randomized studies on individual suggested improvements such as disinfected biopsy needles [154] (S5).

Finally, genomic profiling with next-generation sequencing tools on biopsy and surgical specimens is rapidly developing due to technological advances. Additional features complementing the Gleason score to help tumor grading are evolving, and with existing surgical specimens of high quality together with long follow-up time, the validation and implementation of such tools need not to be far away (S6).

Figure 9: A new diagnostic pipeline for prostate cancer and suggested studies.



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